JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXVII WASHINGTON, D. C., FEBRUARY 9, 1924 NO. 6

SELECTIVE FERTILIZATION IN COTTON[1]

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INTRODUCTION

It has been shown that in cotton, under natural conditions of pollination, a large majority of the ovules are self-fertilized, although the flower is well adapted to cross-pollination and foreign pollen usually reaches the stigmas (*7*,[2] *p. 9, 10, 34, 35*). There is also evidence of complete compatibility, even between different species such as Upland and Egyptian cottons, for pollen of both species proved to be equally effective in accomplishing fertilization when applied separately to the stigmas of Egyptian cotton flowers (*7, p. 40–42*). The question therefore suggests itself whether selective fertilization, in favor of the like pollen when unlike pollen also is present, is a factor in the observed predominance of self-fertilization.

The results of experiments by Balls, by McLachlan and by Kearney, summarized in another paper (*7, p. 42–49*) showed that selective fertilization does occur in cotton. When the stigmas of Egyptian cotton were pollinated simultaneously with approximately equal quantities of Egyptian and of Upland pollen, a large majority of the ovules were found to have been fertilized by the like pollen. The percentage of Egyptian × Upland hybrids resulting from such double pollinations was 10.8 ± 0.6 in an experiment performed by Argyle McLachlan and 18.4 ± 1.5 in an experiment conducted by one of the writers, whereas, if both pollens had been equally effective in accomplishing fertilization, 50 per cent of the resulting plants should have been hybrids.

Yet no general conclusion could be drawn as to the importance of selective fertilization in cotton, because experiments made hitherto in Arizona have not afforded decisive evidence that it occurs in the Upland type also. Reciprocal double-pollinations on Upland cotton by McLachlan indicated little or no selective fertilization, the percentage of Upland × Egyptian hybrids having been 42.3 ± 1.1. Earlier experiments at Sacaton, Arizona, to determine whether selective fertilization takes place in Upland cotton failed because nearly all of the artificially pollinated flowers on the Upland plants were lost by abscission.

The writers, assisted by Max Willett and Dow D. Porter, have now succeeded in obtaining conclusive evidence that selective fertilization, in favor of the like pollen, takes place in Upland, as well as in Egyptian cotton. The purpose of this paper is to describe the experiments which yielded this evidence.

[1] Accepted for publication Dec. 14, 1923.

[2] Reference is made by number (italic) to "Literature cited," p. 340.

Selective fertilization, in favor of like as compared with unlike, although compatible pollen when both are present on the stigmas, has been shown by Jones to occur in maize and tomato (*3, 4, 5, 6*). Experiments of Heribert-Nilsson indicated that it takes place also in Oenothera (*2*). The fact that members of such distantly related families as Poaceae, Malvaceae, Onagraceae, and Solanaceae manifest selective fertilization in favor of the like pollen, points to the conclusion that the phenomenon is by no means uncommon among flowering plants. Its probable importance as a factor contributing to the differentiation of plant forms has been discussed by Jones (*5*).

The evidence for selective fertilization brought forward by Jones, supplemented by data given in this paper, applies only to cases when "germ cells from two individuals of different type are presented at the same time in excess so that not all can fulfill their function" (*4, p. 253*), and has no bearing upon the question whether there is selective fertilization among the several gametes produced by the same individual.

METHOD OF INVESTIGATION

The experiments were conducted at the Cooperative Testing Station at Sacaton, Arizona. The pollinations were made in 1922. The flowers to be pollinated were emasculated in the evening preceding anthesis and were enclosed in paper bags to prevent accidental cross-pollination. The flowers which were to supply the pollen were bagged at the same time. The pollinations were made at 1 p. m. of the following day, the procedure having been to detach the flower supplying the pollen and brush the stigmas of the emasculated flower lightly with the clustered anthers of the staminate flower (*7, Pl. I, IV, V*).

Because of the mechanical difficulty of mixing the two kinds of pollen in equal proportion, recourse was had to the method of double pollination used in an earlier experiment (*7, p. 46*). This consisted in pollinating one-half of the flowers first with the like pollen and then with the unlike pollen and reversing the order of application on an equal number of flowers. The two pollens were applied in as nearly as possible equal quantity and the interval between the two pollinations was negligible.

Two experiments were performed, the pollinations of the first experiment having been made during the period July 21 to 30 and those of the second experiment August 10 to 13. The Egyptian type of cotton was represented in both experiments by the Pima variety. The Upland type was represented in the first experiment by the Lone Star variety and in the second experiment by the Acala variety. The stocks of each variety used in the experiments were not inbred families but had been grown from seed produced under conditions of isolation and were judged to be varietally pure. Well grown plants were used in both experiments, those in experiment 1 having been located in adjacent plats, and those in experiment 2 in the same plat. Two hundred flowers of each variety were pollinated in each experiment.

The seeds produced by the double-pollinated flowers were planted in 1923. As many more bolls matured from the double-pollinated flowers on the Pima than on the Upland plants, the former yielded much greater quantities of seed than the latter.[3] The seeds from each lot of Pima

[3] Abscission of the young bolls is always more pronounced in Upland than in Pima cotton as grown Sacaton.

flowers were therefore mixed thoroughly and a representative sample of each lot was planted. The quantities of seed from the several pollinations of the Upland flowers were so small that all were planted.

The seeds representing each sequence of pollination (like + unlike and unlike + like) on each type of cotton in each experiment were planted as separate populations and these populations were further subdivided, seeds from the upper and the lower halves of the bolls having been planted separately. This gave a total of eight ultimate populations from each of the two experiments, as follows:

Pima × (Pima + Upland)	Upper halves of bolls.
Do	Lower halves of bolls.
Pima × (Upland + Pima)	Upper halves of bolls.
Do	Lower halves of bolls.
Upland × (Upland + Pima)	Upper halves of bolls.
Do	Lower halves of bolls.
Upland × (Pima + Upland)	Upper halves of bolls.
Do	Lower halves of bolls.

These ultimate populations were variously combined in considering the data from different points of view. The plants grown from upper and lower seeds of the bolls from each pollination are treated as separate populations only under the heading "Rate of growth of the pollen tubes in relation to selective fertilization."

Four seeds were planted to the hill and no thinning was done. A total of 2,349 plants was obtained from the double-pollinated flowers of Pima and a total of 1,419 plants from the double-pollinated flowers of the Upland varieties. The difference in number of plants was due to the quantities of seed of the two types available for planting and not to better survival in the germinating and seedling stages of the plants from seeds produced by Pima flowers. For both types of cotton the number of plants which survived averaged 2.6 per hill.

On July 19 and 20, when the plants had developed sufficiently to make recognition of the hybrids unquestionable,[4] counts were made of the total number of plants and of F_1 hybrids in each population and from these data the percentages of hybrids were computed.

COMPARISON OF EGYPTIAN AND UPLAND COTTONS AS TO SELECTIVE FERTILIZATION

The data from the two experiments, showing the evidence for selective fertilization in both types of cotton, are presented in Table I. In each case the results from pollination first with like and then with unlike pollen, and the converse, are combined. It will be shown that the pollen applied first fertilized a greater number of ovules than the pollen applied afterward to the same stigmas, but it seems reasonable to assume that the percentage of hybrids obtained by combining the populations from like + unlike and unlike + like pollinations as one array corresponds with what would have been obtained had it been practicable to mix the two pollens in equal quantity before application to the stigmas.[5]

[4] The characters distinguishing F_1 hybrids of Pima and Upland cotton from the parental types were described in another paper (*8*, *p.* 6, 7).

[5] Nevertheless, a small error is involved owing to the fact that populations of unequal size were obtained from the pollinations like+unlike and unlike+like. If, however, the percentages of hybrids for each sequence of pollination (as given in Table II) are averaged for each variety in each experiment, the averages depart but slightly from the percentages based upon the two sequences taken as one array, as given in Table I, and the significance of the results is not altered.

TABLE I.—*Percentages of F_1 hybrids resulting from pollination of Egyptian (Pima) and of Upland (Lone Star and Acala) cotton flowers with approximately equal quantities of Egyptian and Upland pollen*

Populations from double pollinations of:	Number of—		Percentage of hybrids.
	Plants.	Hybrids.	
EXPERIMENT 1			
Pima with Pima+Lone Star and Lone Star+Pima...	1,381	442	32.0±0.85
Lone Star with Lone Star+Pima and Pima+Lone Star	752	166	22.1±1.02
EXPERIMENT 2			
Pima with Pima+Acala and Acala+Pima.....	968	163	16.8±0.81
Acala with Acala+Pima and Pima+Acala.....	667	220	33.0±1.23
BOTH EXPERIMENTS AS ONE ARRAY			
Double pollinations on Egyptian.....	2,349	605	25.8±0.61
Double pollinations on Upland	1,419	386	27.2±0.80

Considering as one array all plants resulting from double-pollinations of the Egyptian (Pima) flowers and of the Upland flowers, respectively, (Table I, bottom section) it is evident that the percentages of hybrids were practically the same, the difference having amounted to only 1.4 ± 1.0 per cent. In both cases approximately three-quarters of the ovules were fertilized by pollen of the same type and the departures from the 50 per cent expected if there had been no selective fertilization are extremely significant.

It is interesting that while in the first experiment the Pima flowers yielded a significantly higher percentage of hybrids than the Upland flowers (difference 9.9 ± 1.33), in the second experiment it was the Upland flowers that yielded a significantly higher percentage of hybrids (difference 16.2 ± 1.47). The cause of this difference in the results of the two experiments may only be conjectured. Differences in the relative viability of the several pollens would seem to offer the most plausible explanation, but such data as were obtained show practically equal viability.

Pollen from five flowers each of Pima and of Lone Star, each flower having been borne by a different plant, was tested as to its ejection when immersed in a 5 per cent solution of cane-sugar (7, *p. 22–25*). The estimated percentage of grains which ejected was 90 per cent for each of the Pima flowers and ranged from 80 to 90 per cent, with an average of 87 per cent, for the five flowers of Lone Star. The tests were conducted during the period when the double pollinations of experiment 1 were being made. Similar tests of pollen from four flowers of Acala cotton made at the outset of experiment 2, showed ejection of from 90 to 95 per cent of the grains in the field of the microscope.

SEQUENCE OF APPLICATION OF THE TWO POLLENS AS AFFECTING THE PERCENTAGE OF HYBRIDS

Higher percentages of hybrids were obtained when the unlike pollen was applied first than when the like pollen was applied first. The data given in Table II show that this was the case for both types of cotton in

both experiments, although the differences were significant in only two of the four comparisons, that of the Pima flowers in experiment 1 and that of the Upland (Acala) flowers in experiment 2. The differences in these cases were, respectively, 6.3 and 8.4 times the probable error of the difference. The probable explanation is that in some of the flowers the surface of the stigmas was so well covered by the pollen applied first that many of the grains of the other kind of pollen did not come into contact with the stigmatic surface and hence were unable to effect fertilization. It should be noted that the highest percentage of hybrids resulting from application of the unlike pollen first (Acala with Pima + Acala in experiment 2) is significantly lower than the 50 per cent expected if there had been no selective fertilization, the departure having been 7.8 ± 1.76.

TABLE II.—*Percentages of hybrids resulting from double pollination with (a) the like pollen applied first, (b) the unlike pollen applied first*

Sequence of application of the like and unlike pollens.	Number of—		Percentage of hybrids.
	Plants.	Hybrids.	
EXPERIMENT 1			
(*a*) Pima with Pima+Lone Star	741	200	27.0 ± 1.10
(*b*) Pima with Lone Star+Pima	640	242	37.8 ± 1.29
Difference in favor of unlike pollen applied first			10.8 ± 1.70
(*a*) Lone Star with Lone Star+Pima	481	102	21.2 ± 1.26
(*b*) Lone Star with Pima+Lone Star	271	64	23.6 ± 1.74
Difference in favor of unlike pollen applied first			2.4 ± 2.15
EXPERIMENT 2			
(*a*) Pima with Pima+Acala	627	100	15.9 ± 0.98
(*b*) Pima with Acala+Pima	341	63	18.5 ± 1.42
Difference in favor of unlike pollen applied first			2.6 ± 1.73
(*a*) Acala with Acala+Pima	309	69	22.3 ± 1.59
(*b*) Acala with Pima+Acala	358	151	42.2 ± 1.76
Difference in favor of unlike pollen applied first			19.9 ± 2.37

SELECTIVE SURVIVAL AS A FACTOR AFFECTING THE PERCENTAGES OF HYBRIDS

The percentage of hybrids in an adult population from double-pollinated flowers would not represent the actual degree of selective fertilization if there had been selective survival in favor of either the homozygotes or the heterozygotes. It is possible, even, that what would appear to be a case of selective fertilization in favor of like pollen might be, in reality, merely a result of pronounced selective survival of the homozygotes. It is important, therefore, to examine critically the evidence as to whether there had been selective survival in the experiments under discussion.

Whether selective survival took place during germination and in the early stages of seedling growth is the first question to be examined. Had this been the case it seems probable that the heterozygotes rather than the homozygotes would have benefitted, since the Pima-Upland hybrid plants soon become conspicuously larger and more vigorous than the Pima or the Upland plants resulting from fertilization by like pollen. If there

had been selective survival of the heterozygous seeds or seedlings, the percentage of hybrids in the adult population would have been higher than the percentage of ovules fertilized by the unlike pollen. In that case the data in Table I would indicate less than the full measure of selective fertilization in favor of the like pollen.

Four seeds were planted in each hill but in many of the hills only one or two plants survived. If there had been selective survival in favor of either the homozygotes or the heterozygotes, it seems probable that the percentage of hybrids obtained where conditions were relatively unfavorable (hills containing one or two plants) would differ from the percentage obtained where conditions were more favorable (hills containing three or four plants). The percentages of hybrids under both conditions were therefore computed for the populations resulting from the double-pollination of Pima and of Upland flowers, respectively, and are given in Table III.

TABLE III.—*Percentages of hybrids in hills containing 1 or 2 and in hills containing 3 or 4 plants, respectively, four seeds having been planted in each hill*

Number of plants per hill.	Percentages of hybrids in populations resulting from double pollination of—			
	Pima flowers.		Upland flowers.	
	Total plants.	Percentage of hybrids.	Total plants.	Percentage of hybrids.
1 or 2	676	23.6±1.10	411	29.6±1.52
3 or 4	1,657	26.6±0.73	998	26.3±0.94
Difference	.....	3.0±1.32	.....	3.3±1.79

The data presented in Table III show that in the population from double-pollinated Pima flowers the percentage of hybrids was greater where the conditions were more favorable to survival, while in the population from double-pollinated Upland flowers the converse was true. In neither population, however, was the difference significant, and it may be concluded that the percentages of hybrids obtained in these experiments were not appreciably affected by conditions operating after the seeds were planted.

The evidence seems conclusive that the case under consideration can not be accounted for as one of selective survival in the germinating and seedling stages. It remains to consider whether selective survival may have occurred at the time of fertilization or immediately thereafter. Since in the adult population homozygotes were much more numerous than heterozygotes, it might be assumed that even if equal numbers of the female gametes had been reached by both kinds of male gametes, an undue proportion of those reached by the unlike male gametes had perished because fertilization was not completed or because the resulting zygotes were unable to develop. Direct evidence on this point would be very difficult to obtain but the following considerations make it improbable that selective fertilization in this material can be explained in this way.

The mean number of ovules in the ovary of Pima cotton, as determined in 1922, was 21.5±0.10. The mean number of seeds in the bolls which

developed from the double-pollinated Pima flowers in the experiments here described was 16.8 ± 0.14. Therefore, an average of 4.7 ovules had failed to develop into seeds. The proportion of hybrids in the adult population from seeds borne by Pima plants, taking the populations of the two experiments as one array, was 25.8 per cent (Table I). It is computed therefore that the mean number of heterozygous seeds per boll was 4.33 (25.8 per cent of 16.8). Assuming that all of the ovules which failed to develop represent heterozygotes or uncompleted heterozygous unions and adding the mean number of undeveloped ovules to the mean number of heterozygous seeds, a total of 9.03 is obtained as representing the mean number of possible heterozygotes per boll. Even this number is only 42 per cent of the mean number of ovules. A similar computation in regard to the Upland varieties in these experiments is impracticable because satisfactory data as to their mean numbers of ovules are not available.

There is, however, no reason to assume that all or most of the undeveloped ovules represent unsuccessful unions or attempts at union with unlike male gametes. The mean number of seeds per boll from the double-pollinated Pima flowers of these experiments (16.8) exceeds the average (16.5), for 10 lots of bolls from naturally pollinated flowers of the same variety, as given in another publication (7, *p. 51*, *Table 30*). The highest mean number for any of these lots was 18.6 or 2.9 fewer than the mean number of ovules. It is evident that under the most favorable conditions a number of the ovules fail to develop, probably because they are defective or because they are not reached by pollen tubes. The conclusions seem warranted, therefore, that the percentage of undeveloped ovules in the present experiments was not abnormally high and that there had been no selective survival at fertilization or in the early stages of development of the zygote.

RATE OF GROWTH OF THE POLLEN TUBES IN RELATION TO SELECTIVE FERTILIZATION

In seeking an explanation of selective fertilization, the possibility of a difference in the rapidity of germination and of pollen-tube development of the like and unlike pollens is the first point to be considered. An experiment was described in another paper (7, *p. 42*) in which it was sought, by excising the stigmas and style at successive intervals of time after pollination, and by comparing the degrees of fertilization thus attained, to determine the relative rates of growth of Pima and of Upland pollen deposited separately on the stigmas of different Pima flowers. The growth rates of the like and unlike pollens, under these conditions, were found to be very similar. The experiment was repeated in 1923, a number of Pima flower buds having been emasculated the evening before anthesis and pollinated at 8 a. m. the next day, some of the flowers with Pima pollen and others with Upland pollen (Acala variety). The styles of equal numbers of flowers of each pollination were then severed at the summit of the ovary at intervals of 8, 10, and 12 hours after the pollen was deposited.[6] A record was kept of the number of bolls which developed from each lot of flowers and of the number of seeds in each boll. The results are summarized in Table IV.

[6] This method was used by Heribert-Nilsson (2) in an investigation of the rate of pollen-tube growth in Oenothera. His results showed a more rapid growth of the like pollen.

TABLE IV.—*Relative rapidity of penetration of the ovary of Pima cotton by Pima and by Upland pollen tubes, as measured by the degrees of fertilization attained when the pistils were excised at successive intervals*

Number of hours from pollination to excision of the pistils.	Pollination with—					
	Pima pollen.			Upland pollen.		
	Flowers treated.	Percentage of bolls developed.	Mean number of seeds per boll.	Flowers treated.	Percentage of bolls developed.	Mean number of seeds per boll.
8.....	100	0	0	100	0	0
10.....	100	2. ±0. 94	8. 5±3. 58	100	8. ±1. 83	11. 5±1. 42
12.....	100	10. ±2. 02	5. 3±1. 06	100	18. ±2. 59	7. 8±0. 90

In Pima cotton, under normal conditions of pollination, from 75 to 97 per cent of the flowers develop bolls (7, *Table 34, p. 55*) and the bolls contain an average of from 14 to 18.5 seeds (7, *Table 30, p. 51*). Comparing with these figures the percentages of bolls developed and the mean numbers of seeds per boll as given in Table IV, it is evident that relatively few pollen tubes had penetrated the ovaries within 12 hours after deposition of the pollen. Yet, it should be noted that of the flowers excised at this time, one, pollinated with Pima pollen, developed a boll containing 18 seeds and one, pollinated with Upland pollen, developed a boll containing 20 seeds.

The data, so far from showing more rapid development of the like pollen, seem to indicate that better fertilization was attained by the unlike pollen when the time available for penetration of the ovary was shortened by excision of the pistil. In fact, however, the differences between the pollinations with Pima and with Upland pollen, in percentages of bolls and in mean number of seeds, for either the 10-hour or the 12-hour period, are in all cases less than three times the probable error of the difference.

The results of this experiment, agreeing in the main with those of a similar experiment previously reported, make it reasonably certain that, when separately applied, like pollen has no advantage over unlike pollen in its rate of penetration of the ovary of Pima cotton. It does not follow, however, that there may not be a difference in the rate of penetration when both pollens are present on the stigmas of the same flower. Jones (6) obtained evidence that in maize, when a mixture of two pollens was applied to the stigmas, there resulted a greater proportion of cross-fertilization in the upper than in the lower half of the ear, from which he inferred a slower rate of development of the unlike pollen. The indicated differences in rate of growth seem, however, too small to account for the degrees of selective fertilization shown in some of Jones' combinations.

Maize, with its elongated female inflorescence and extraordinarily long styles, is far superior to cotton as a subject for the study of differential fertilization. In Pima cotton the stigmas and styles average only 30 to 35 mm. in length and in the Upland varieties used in these experiments they average not more than two-thirds as long as in Pima. The ovary of both types is only 5 or 6 mm. long at anthesis. In view of these facts it seemed unlikely that evidence of differential fertiliza-

tion could be obtained with this material, but it was thought advisable to test the possibility by experiment.

In comparing cotton with maize in respect to differential fertilization, the distinction applies which was made by Jones in comparing the results of Correns with Melandrium and his own results with maize. "In the former the pollen tubes enter the ovary at a common point and are free to fertilize the first ovules they reach. The tubes which grow fastest, therefore, fertilize the ovules in the upper part of the ovary, leaving the slower-growing tubes to pass on down to the lower part. In Zea each ovule has a separate style, so that the longer the distance to traverse is, the less chance will the slower-growing tubes have of reaching the goal first" (*6, p. 173*). Since cotton resembles Melandrium rather than Zea in these points of structure, we should expect to find a greater proportion of cross-fertilized ovules in the lower than in the upper part of the ovary, if the tubes of the like pollen grow more rapidly than the unlike pollen tubes.

All bolls resulting from the double-pollination of Pima and Upland flowers in the two experiments described in the first part of this paper were halved when ripe and the seeds from the upper and from the lower halves were planted separately. (See p. 331.) The per centages of hybrids from upper and from lower seeds are stated in Table V, the populations from pollination with like+unlike and with unlike+like pollen having been taken as one array for each type of cotton in each experiment.

TABLE V.—*Percentages of hybrids in populations grown from seeds from the upper and from the lower halves of bolls produced by double-pollinated flowers of Pima and of Upland cotton*

Experiment and pollination.	Half of boll furnishing seed for planting.	Number of—		Percentage of hybrids.
		Plants.	Hybrids.	
EXPERIMENT 1				
Pima with Pima and Lone Star	Upper	700	227	32.4±1.19
Do	Lower	681	215	31.6±1.20
	Difference			0.8±1.69
EXPERIMENT 2				
Pima with Pima and Acala	Upper	405	63	15.6±1.21
Do	Lower	563	100	17.8±1.09
	Difference			2.2±1.63
EXPERIMENT 1				
Lone Star with Lone Star and Pima.	Upper	373	86	23.0±1.47
Do	Lower	379	80	21.1±1.41
	Difference			1.9±2.04
EXPERIMENT 2				
Acala with Acala and Pima	Upper	384	123	32.0±1.60
Do	Lower	283	97	34.2±1.90
	Difference			2.2±2.48

The data given in Table V show in no case a significantly greater percentage of hybrids from seeds contained in the lower halves of the bolls than from seeds contained in the upper halves, and therefore afford no evidence that the rate of growth of the tubes of the unlike pollen is inferior to that of the like pollen when the two kinds of pollen are in direct competition.

The pistils of the Upland varieties are much shorter than the Pima pistils, but the Upland pollen fertilized equally well the ovules in the lower and in the upper half of the Pima ovary. Hence there is no evidence of a correlation between the length of the pistil and the length attainable by the pollen tubes, such as has been observed in other plants.[7]

DISCUSSION

Evidence has been presented by one of the writers that a large majority of the ovules normally are self-fertilized in both the Egyptian and the Upland type of cotton (*7, p. 9, 10*). It was also shown that pronounced selective fertilization occurs in the Egyptian type, but no conclusion could be drawn as to the general importance of this phenomenon as a factor in the observed preponderance of self-fertilization, owing to lack of conclusive evidence that selective fertilization takes place in the Upland type also (*7, p. 42–49, 64*). The results of experiments described in the present paper make it clear that selective fertilization occurs in both types and in about the same degree. Nearly 75 per cent of the ovules, on the average, are fertilized by pollen of the same type and variety when pollen of the other type is present on the stigmas simultaneously and in approximately equal quantity.

This evidence of pronounced selective fertilization should be considered in connection with the evidence given in another paper (*7, p. 34, 35, 63*) that as a rule a majority of the pollen grains reaching the cotton stigmas have originated in the same flower, in other words that there is usually present on the stigmas an excess of self-pollen over foreign pollen.[8] These findings afford a satisfactory explanation of the fact that self-fertilization predominates in cotton although the flower is so admirably adapted to cross-pollination (*7, p. 12*).

Egyptian cotton, although apparently not referable to any one of the species of Gossypium recognized by taxonomists, is very closely related to *G. barbadense* L. and to *G. peruvianum* Cav. and exhibits differences from Upland cotton (*G. hirsutum* L.) which are of specific if not subgeneric magnitude (*8, p. 4–6, Plates I, III, V–XI*). Nevertheless, the results of experiments described in another paper (*7, p. 40–42*) have shown that when Upland pollen alone was applied to the stigmas of Pima flowers the fertilization attained was at least equal to that resulting from the application of Pima pollen alone. It was found also that the reciprocal pollinations on Upland showed only a slight

[7] McClelland (*9*) ascertained that when two species of Vanilla, one of which had a relatively short and the other a long column, were cross-pollinated, pollen of the first type fertilized few or none of the ovules near the base of the ovary of the second type, while pollen of the second type (long column) fertilized the basal ovules of the first type (short column) even more readily than did its own pollen. On the other hand, Tokugawa found that when two species of Lilium, one having a long style and the other a short style, were reciprocally cross-pollinated, the pollen tubes of either species grew faster in the pistil of the same species than did the foreign pollen tubes (*10, p. 29, Table XII*).

[8] The seeming discrepancy that under natural conditions more of the ovules appear to be self-fertilized in Egyptian than in Upland cotton, although selective fertilization occurs in both types in practically the same degree, is removed by the observations previously recorded (*7, p. 34–36*) that the Upland flowers usually receive foreign pollen in greater quantity than the Egyptian flowers.

and possibly not a significant advantage in favor of the like pollen. The two kinds of pollen, therefore, do not differ appreciably in compatibility.

Tests made in sugar solution showed no important differences in the viability of the several pollens, and the very fact that the Egyptian and the Upland flowers, when pollinated with a mixture of pollen of the two types, yielded approximately equal mean percentages of hybrids indicates that the average viability of the two kinds of pollen was practically the same.[9]

The case of selective fertilization here described is clearly not attributable to differences in the viability or in the compatibility of the two kinds of pollen. The evidence is against the assumption that the phenomenon is one of selective survival of the homozygotes, at any stage from the formation of the zygote to the attainment of the adult state. So far as could be ascertained, the unlike pollen is not inferior to the like pollen in the rate of development of its tubes and in ability to unite with the female gametes. How, then, is the fact of selective fertilization in cotton to be explained?

The only hypothesis which seems to fit the observed facts is one previously advanced (*7, p. 48*) that the presence of like pollen in some way prevents the germination or subsequent development of many of the unlike pollen grains when both kinds are present on the stigmas. That the inhibiting factor does not reside in the stigmas themselves when like pollen is absent seems clear from the fact that when applied separately the unlike pollen is not inferior to the like pollen in rapidity of development and ability to effect fertilization.[10]

It is conceivable, however, that the presence of pollen of the same type may induce a physiological reaction in the stigmas which makes them a relatively unfavorable medium for the germination or growth of pollen of a different type. The further assumption must be made that, in spite of this unfavorable condition, some of the unlike pollen grains are able to accomplish fertilization, possibly because they are more resistant, possibly because they happen to be so placed as to avoid the tracts of stigmatic tissue affected by contact with the like pollen. It would seem that such of the unlike pollen grains as succeed in avoiding or overcoming this obstacle develop their tubes as rapidly as do the pollen grains of the same type, and that there is no appreciable difference in the readiness with which the two kinds of male gametes unite with the female gametes.

The double-pollination experiments with maize carried out by Jones involved pairs of types which showed various degrees of genetic difference, some being nearly and others very remotely related. A pronounced positive correlation was found to exist between the degree of selective fertilization in favor of like pollen and the genetic distinctness of the types, as indicated by the degree of heterosis manifested by the crosses between them. As Jones expresses it, "the handicap placed upon the foreign pollen is proportional to the germinal unlikeness" (*4, p. 283*). The experiments with cotton described in this paper involve only unrelated types which differ profoundly in their morphological characters.

[9] It is, of course, possible that, even with a difference in the pollen viability of two varieties, equal percentages of hybrids might result from reciprocal double-pollination if selective fertilization in favor of the like pollen were more pronounced in the variety having the less viable pollen.

[10] This fact is also difficult to reconcile with the suggestion made by Jones (*4, p. 284, 285*) that we are concerned here with a phenomenon analogous to the toxicity of foreign proteins.

Very pronounced heterosis is shown by hybrids between these types, yet the degree of selective fertilization in this case was much smaller than in some of the maize combinations tested by Jones, in which, when double-pollinated, there was a near approach to cross-sterility. It remains to be ascertained whether Gossypium is analogous to Zea in showing a smaller degree of selective fertilization between more nearly related forms.[11]

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[11] The results of experiments made by Balls (*1, p. 122–125*) indicate that the analogy exists, for he found that when either Egyptian or Upland cotton was pollinated with a mixture of pollen of the same type and pollen from Egyptian×Upland F_1 plants, the percentage of hybrids resulting was much higher than when a mixture of Egyptian and Upland pollens was applied to the stigmas. This is in conformity with Jones' findings in Zea, since the first generation hybrid is, of course, more nearly related to either parent than is the other parent.

THE EFFECT OF FERTILIZERS ON THE DEVELOPMENT OF STEM RUST OF WHEAT[1]

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INTRODUCTION

For many years there has been an opinion that the severity of rust attacks on wheat and other cereals was influenced profoundly by available soil nutrients. Especially was it thought that heavy fertilization with nitrogenous manures was conducive to abundant development of rusts. Most farmers, and many scientists, did not distinguish between the different kinds of rusts, so that many of the early observations have but little value. About ten or twelve years ago, however, many farmers in Minnesota and neighboring States asserted that the kind of soil on which wheat was grown, as well as the amount of barnyard manure applied, affected very greatly the development of stem rust, *Puccinia graminis* Pers. In some localities there also was a general impression that wheat grown on clover soil was very likely to be injured badly by the rust. These observations seemed to indicate that nitrogenous fertilizers predisposed wheat to attack by *Puccinia graminis*, and the results of controlled experiments made by several European investigators apparently supported these views.

Comes (*7*)[3], Spinks (*30*), Hiltner (*12*), Stranak (*33*) and others published the results of experiments which indicated that soil fertilization influenced profoundly the severity of stripe rust, *Puccinia glumarum* (Schm.) Erikss. and Henn., on wheat. Experiments conducted by the senior author (*31*) show that in general the absence or presence, in excessive amounts, of various nutrient substances, such as nitrogen and phosphorus salts, did not directly affect the immunity or susceptibility of wheats to *P. graminis*. Conditions favoring normal host development increased the vigor of the rust. It seemed desirable to make more extensive experiments in the field, on different types of soil, and over a period of several years. Empirical experiments of a practical nature therefore were begun in 1914 and were continued for eight years. The writers tried to ascertain facts, the explanation of which was sought in special investigations.[4]

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[1] Accepted for publication Nov. 19, 1923. Cooperative investigations between the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agriculture of the University of Minnesota. Published with the approval of the Director, as Paper No. 293 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writers are greatly indebted to Dr. F. J. Alway, Chief of the Division of Soils, Department of Agriculture, University of Minnesota, for advice and assistance and for placing the Crookston plats at their disposal; and to Dr. C. H. Bailey, head of the Section of Cereal Technology, Division of Biochemistry, University of Minnesota, for making certain analyses. They also are indebted to John H. Parker, F. J. Piemeisel, A. G. Newhall, and G. R. Hoerner, formerly of the Office of Cereal Investigations, and to C. R. Hursh, Agent, Office of Cereal Investigations, and Assistant Pathologist, Department of Agriculture, University of Minnesota, for assistance in taking notes.

[3] Reference is made by number (italic) to "Literature Cited," p. 377.

[4] Dr. Freeman Weiss and Dr. C. R. Hursh made these studies as a part of the cooperative project between the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agriculture of the University of Minnesota. The results will be published in separate papers.

REVIEW OF PREVIOUS WORK

A summary of what has been published on the factors which predispose to or protect plants from disease would comprise many bulky volumes. It should be axiomatic that different host plants and pathogenes are affected differently by environmental conditions. It is difficult or impossible, therefore, to establish universally applicable principles, and, for this reason, only the most relevant literature is reviewed in this paper.

Little (*15*), in 1883, stated that low, rich soils and heavy manuring, especially with nitrogenous manures, predisposed wheat to rust. Anderson (*1*), in 1890, and Bolley (*5*), in 1889, expressed a similar opinion. Anderson found hard, flinty wheats resistant to rust. He thought resistance due possibly to the large amount of silica in such plants, and advised against the use of excessive quantities of nitrogenous fertilizers. He recommends that salt, iron sulphate, and lime be used to prevent rust.

Petermann (*22, p. 15–16*) reported in 1902 that wheat manured with superphosphate was very badly rusted, while that fertilized with volcanic slag was almost free. He thought the difference in resistance was due to differences in the strength of the cell walls.

In 1903, Ward (*38*), showed lack of minerals did not confer immunity from *Puccinia dispersa* Erikss. on bromes.

Remer (*24*) observed in 1903 the effect of fertilization on cereal rusts in Silesia. He concluded that even a slight excess of nitrogen predisposed cereals to rust. Stable manure applied to soil previously devoted to clover predisposed wheat and oats especially. He thought that excessive vegetative vigor, consequent lodging, and greater leaf surface favored the development of rust, and that acid phosphate usually inhibited its development on plants grown in soils in which there was not too much nitrogen. Potash did not predispose his cultures to rust.

McAlpine (*16*), in 1906, found that the most vigorous plants often were most severely rusted and that early maturity favored escape from rust injury. Johnson (*14*), in 1911, stated that wet soils and luxuriant growth predisposed timothy to the timothy rust, *P. graminis phleipratensis* Erikss. and Henn. Montemartini (*20*) stated that starved plants were immune from rust and that phosphates always increased resistance. Freeman and Johnson (*8*) cite general observations on the apparent predisposing effect of nitrogenous fertilizers.

Biffen (*4*), in 1912, found *P. glumarum* most abundant on plants which had received a complete fertilizer. The amount of rust decreased with a decrease in the amount of fertilizer used.

Comes (*7*) states that nitrogen predisposed plants to rust, while non-nitrogenous fertilizers, especially potash, tended to increase resistance. He found that the concentration of organic acids was lowered, and that resistance to infection was proportionately lowered.

Spinks (*30*) studied the effect of nutrient salts on the susceptibility of wheat to *P. glumarum* and *Erysiphe graminis* DC., and of barley to *E. graminis*. He concluded that heavy applications of nitrogenous fertilizers increased the susceptibility of wheat to stripe rust, and of wheat and barley to powdery mildew. Potassium salts increased the resistance but did not counteract the effect of large amounts of nitrogen. Spinks concludes, however, that a highly resistant variety tends to remain resistant even when heavily fertilized with nitrogenous manures. Voelcker's results (*37*) were essentially similar to those of Spinks.

Hiltner (*12*), in 1914, noted that excessive use of Chile saltpeter greatly increased the amount of rusts on fall-sown wheat, but excessive amounts of ammonium sulphate did not increase them as much.

In 1915, Stranak (*33*), in his investigation of the effect of fertilizers on infection of wheat by *P. glumarum*, observed the greatest injury when the soil fertilization was unbalanced. Wheat grown on insufficiently fertilized soil suffered the greatest damage. But wheat overfertilized with nitrogen, especially when there was an insufficient supply of potash or phosphates, also was nearly always severely rusted.

Molz and Müller (*21*) in 1914 and 1916 concluded that potassium and phosphate fertilizers increased the resistance of wheat to stripe rust, and that the effect of nitrogen was uncertain.

Stakman (*31*) in 1913 made experiments on the effect of nutrient salts on the development of *P. graminis* on wheat plants in soil and sand cultures and found that excessive amounts of such fertilizers as nitrogen and phosphorus salts had no direct effect on the immunity or susceptibility of wheats. He considered temperature conditions and atmospheric humidity probably more important than soil conditions.

Armstrong (*2*), in 1922, pointed out that wheat fertilized with sodium nitrate was delayed and opportunity for the development of *P. glumarum* was increased. He attributed the effect of nitrogen to a lengthening of the growing period rather than to an actual increase of susceptibility.

Gassner (*10*), investigating the effect of fertilizers on the development of rusts of wheat, oats, barley, rye, and maize, in Uruguay, found that an apparent effect of fertilizers on the development of *P. graminis* was due to the effect on maturity of the host plants rather than to a change in the actual resistance.

Vavilov (*36, p. 60–61*) concluded that the apparent increased susceptibility of wheat to *Puccinia triticina*, when grown in soil fertilized with nitrogen, was due to increased development of leaf surface rather than to any change in real resistance. He grew 30 pure-line selections of spring wheat, of varying degrees of resistance to *P. triticina*, in unfertilized soil and also in soil heavily fertilized with potassium nitrate. There were only slight differences in the amount of rust on the plants in different plats, and resistant varieties remained resistant under all conditions.

Raines (*23*) studied the factors governing the virulence of *P. coronata* Cda., *P. secalina* Grove, *P. triticina* Erikss., and *P. sorghi* Schw. He was of the opinion that "a more catholic point of view in pathologic thought, recognizing that, for longer or shorter phases in the course of a disease, the relation between host and parasite may be highly mutualistic, would be of material value as a working concept in the study of diseases and in defining the practical problem of disease prevention and control."

This conclusion, in general, corroborates the evidence given by Ward (*39*), Arthur (*3*), Sheldon (*27*), Gibson (*11*), Stakman (*31*), Fromme (*9*), Mains (*17*), and others that the vigor of various rust parasites is likely to be directly proportional to the vegetative vigor of the host. The observations of Zavitz (*41*) on the effect of spacing also may be explained on the basis of greater vegetative vigor. Butler (*6, p. 473–474*) gives some evidence, however, that well nourished coffee bushes can withstand attacks by coffee rust, *Hemileia vastatrix* B. and Br., better than those which are growing under less favorable conditions.

It is apparent that the general consensus of opinion is that nitrogenous fertilizers increase the susceptibility of cereals to rusts, while potas-

sium and phosphorus salts tend to decrease it. Some investigators, notably Comes, apparently attribute the effect principally to changes in the chemical composition of the plants; others, such as Schindler (*26, p. 168*), Russell (*25*), and Miyake and Adachi (*19*) think the effect is rather on the mechanical composition of the cell walls. Schindler says that acid phosphates seem to cause the development of stronger tissues ("strammeres Gewebe"). Russell concludes that the walls of plants fertilized with excess nitrogen are thinner than they normally would be, and parasitic fungi therefore can penetrate more easily. Miyake and Adachi agree with Russell regarding the effect of nitrogen, and they report that potassium fertilizers strengthen the cell walls and thus increase resistance. Still others, especially Gassner (*10*), Freeman and Johnson (*8*), Stakman and Aamodt (*32*), and Armstrong (*2*), suggest that the effect of fertilizers is at least partly indirect.

Hurd's results (*13*) do not support the views of Comes that acidity of the host plant is the determining factor in resistance. She found no positive correlation between hydrogen-ion concentration and resistance. Neither could Hursh [5] find any correlation between sugar content of different varieties and the resistance to *Puccinia graminis*. On the other hand, there is some evidence that fertilizers may alter the structure of the host sufficiently to account partly, at least, for differences in the resistance of the same variety grown under different conditions.

That fertilizers may cause an apparent change in resistance by influencing rate of growth, density of stand, date of maturity, and yield can hardly be questioned.

OBJECTS OF THE INVESTIGATION

The objects of the investigation were: 1. To ascertain whether natural and artificial fertilizers alone and in various combinations, directly or indirectly, affect the degree of susceptibility of susceptible and resistant varieties of wheat to *Puccinia graminis* and *P. triticina;* 2. To ascertain what effect fertilizers have on the density of stand, the succulence of the plants, the degree of lodging, the date of maturity and similar characters which might indirectly influence the development of the rust; 3. To determine whether the same amount of rust will produce different degrees of injury when the plants are grown in different types of soil; 4. To determine whether plants receiving excessive nitrogen fertilization are especially liable to rust injury.

Attempts were made to predispose plants by means of barnyard manure, by growing them on clover and alfalfa soils and by adding nitrates directly to the soil. It was desired also to find out whether phosphates and potash could counteract the effect of large amounts of nitrogen and whether a combination of fertilizers could be found which would enable the plants to yield well in spite of rust. While the primary object was to determine, for practical purposes, the effect of normal amounts of fertilizers on the severity of rust attacks, attempts also were made to ascertain the effect on rust resistance of excessive amounts of fertilizers. As the work progressed, it became increasingly evident that it would be necessary to secure data on the effect of fertilizers on density of stand, succulence, degree of lodging, date of maturity and similar characters which indirectly might affect the development of rust without necessarily changing the actual resistance of the plants.

[5] Unpublished results.

After data had been obtained for several years from the field experiments, it became evident that the same amount of stem rust might not equally injure plants grown in different kinds of soil. It was suggested that plants very heavily fertilized with nitrogen might be especially liable to rust injury on account of succulence, weak straw, and high water requirement. Special investigations therefore were made on these phases of the problem.[6]

EXPERIMENTAL METHODS

Field experiments and observations were made for eight years on several types of soil and in several localities in Minnesota. Some pot experiments also were made. Four of the years were "rust years" and four were not.

The most extensive experiments were made on University Farm, and the Quinn farm, St. Paul. One series was made at Anoka; and observations were made on the regular fertilizer plats of the Division of Soils at Morris and at Crookston.

The soil on University Farm is the Hempstead silt loam. It varies considerably, but, in general, is well supplied with all necessary plant nutrients. It is described by Smith and Kirk (*28*) as follows: "The surface soil of the Hempstead silt loam consists of about 10 to 18 inches of black to dark brown silt loam, underlain by a subsoil consisting of brown to yellowish-brown-silty clay to silty clay loam, which extends to a depth of about 3 feet. Local variations include a somewhat open and loamy texture on the one hand, and a rather heavy and compact structure on the other. The substratum consists of a bed of rather clean gravel and sand." The series at University Farm was on the heavier and compact type, while that on the Quinn farm was on the more open and loamy texture.

The soil at Anoka is a loamy sand, often deficient in nitrogen. The following description is given by Smith et al. (*29*): "The Merrimac loamy sand consists of a brown to very dark brown loamy sand, 10 to 18 inches deep, underlain by a brown loamy sand subsoil and substratum."

The plats at Crookston were on loam which is very rich in organic matter and does not require artificial fertilizers. According to Mangum et al. (*18*), "The soil, to an average depth of 12 to 15 inches, consists of a dark brown to black silty clay which contains a very large percentage of organic matter. This material becomes slightly heavier as the depth increases and grades at about 12 inches into a heavy drab to gray silty clay, with a very finely stratified structure."

The fertilizer plats were laid out on uniform, level soil in order to avoid errors due to lack of uniformity in chemical composition, and to the washing of fertilizers from one plat to another. During the first four years, the size of the plats was one square rod. Three-foot alleys separated them from each other. In 1918, 1920, 1921, and 1922 observations were made on tenth-acre permanent fertilizer plats. Data on percentage of stem rust only were obtained from these plats.

The general plan of the commercial fertilizer plats in 1915, 1916, and 1917 on University Farm is shown in Table I. Modifications of this plan are indicated in the proper places.

[6] The results are published in the Journal of Agricultural Research in separate papers by Dr. Freeman Weiss and Dr. C. R. Hursh.

TABLE I.—*General plan of commercial fertilizer plats in 1915, 1916, and 1917 on University Farm, St. Paul, Minn.*[a]

Fertilizer.		Fertilizer, kind and amount in pounds per acre.						
Kind.	Amount per acre (pounds).	Sodium nitrate.			None.	Potassium chlorid.		
		1,000	500	250		1,000	500	250
Acid phosphate.....	2,000	1	2	3	4	5	6	7
	1,000	8	9	10	11	12	13	14
	500	15	16	17	18	19	20	21
	None.	22	23	24	25	26	27	28

[a] The plats are numbered from left to right in all plat plans.

It will be noted from Table I that there were 28 plats in the regular series. The fertilizers, and amounts applied, are indicated at the left of the horizontal columns and at the tops of the vertical ones. For instance, plat 3 (reading from the upper left-hand plat to the right), received an application of acid phosphate at the rate of 2,000 pounds per acre and sodium nitrate at the rate of 250 pounds per acre. Plat 4 received only phosphate, and plat 25 is a control. Each of the three fertilizers was used alone in three concentrations, and combinations were made between acid phosphate and each of the other fertilizers. This plan was changed slightly in some of the experiments in order to make other combinations of fertilizers, and, on the poorer soils, a complete fertilizer also was used. In addition, experiments were made with barnyard manure in combination with other fertilizers. In 1915, plats were established at University Farm on cabbage, clover, and alfalfa soils. The details will be given under each experiment, as they vary considerably.

The principal commercial fertilizers used in the tests made during the first four years were acid phosphate, sodium nitrate and potassium chlorid. In 1916, potassium chlorid could not be obtained, so potassium sulphate was used instead. The barnyard manure used was well rotted.

The acid phosphate was worked thoroughly into the soil before seeding, while potassium chlorid and potassium sulphate were applied as a top dressing immediately after seeding. The sodium nitrate was applied as a top dressing shortly after the plants had emerged from the soil, and applications were made at two-week intervals, at the rate of 250 pounds per acre, until the total amount had been applied to each plat. The manure, which always refers to well-rotted barnyard manure, was applied before seeding and was thoroughly worked into the soil.

A resistant and a susceptible variety of wheat were sown in each plat in most of the experiments at University Farm. The varieties used and their average degree of resistance to stem rust under field conditions will be given under the description of each experiment.

Under ordinary conditions a severe epidemic of stem rust is not sure to occur every year. Owing to location, no attempt was made to induce the development of artificial epidemics on the wheat in the plats on the Quinn farm, or at Anoka and the Crookston substation. Definite measures, however, were taken to produce a severe epidemic on the

plats at University Farm. By the method described below a severe epidemic was obtained each year.

Common barberry bushes, covered with wheat straw containing teliospores, were grown in the field. This straw was placed around the barberry bushes late in the fall and was allowed to remain until the leaves had unfolded in the spring. Abundant aecia developed on the barberry leaves. Winter wheat was sown early in the fall around the barberry bushes. This resulted in the production of urediniospores on the winter wheat early in the spring. The wheat plants in the fertilizer plats were then sprayed with a water suspension of aeciospores from the barberry leaves and urediniospores from the winter wheat. The plants were sprayed every other day after sunset, or during the day in wet, cloudy weather, until a heavy epidemic was assured. In addition, flower pots, containing wheat seedlings, well covered with uredinia, were placed in the plats. Large quantities of rusted material were developed in the greenhouse and there held in readiness to be placed in

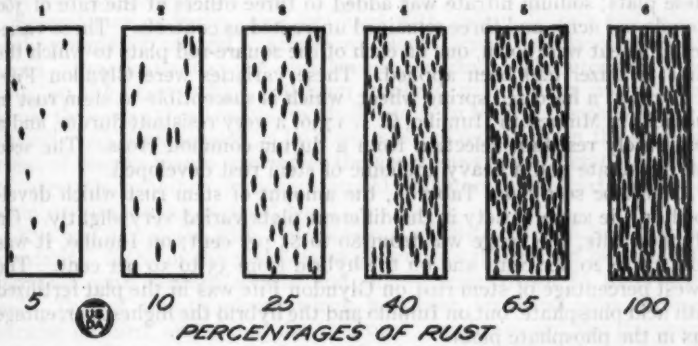

FIG. 1.—Scale for estimating percentage of stem rust. The shaded spots represent rust, and the figures represent approximately the rust percentages computed on the basis of the maximum amount of surface covered by rust as shown in No. 6, which represents 37 per cent of surface covered with rust pustules and is arbitrarily selected as 100 per cent. Other percentages are based on No. 6. (From Office of Cereal Investigations, United States Department of Agriculture.)

the field at the proper time. Spore suspensions for spraying also were made from this material. Hand inoculations were made in the field in the same manner as in the greenhouse. For this purpose control rows of a susceptible variety were sown along the side of the plat. After the inoculum had been placed on the seedlings, they were covered with a bell jar, or inverted flower pot, for 48 hours, the soil being kept wet around the jar or pot during this period.

The scale (fig. 1) used in the stem-rust investigations made by the Office of Cereal Investigations of the United States Department of Agriculture, was used in estimating the percentage of rust.

The final percentages of rust infection were estimated just before the plants ripened. They were made by several experienced observers, some of whom did not know the disposition of fertilizers in the plats. The estimates made by the different individuals did not vary over 5 per cent for any one plat.

Estimates of the degree of lodging, the severity of stem rust on the peduncle, sheath and leaves, the amount of seed shriveling, and the

degree of crinkling are indicated in the tables of results by the following figures:

0 per cent = none.
20 per cent = very light.
40 per cent = light.
60 per cent = medium.
80 per cent = heavy.
100 per cent = very heavy.

EXPERIMENTAL RESULTS

EXPERIMENTS WITH COMMERCIAL FERTILIZERS IN 1914

The experiments in 1914 were preliminary. Nine plats of 1 square rod each, separated from each other by 3-foot alleys, were laid out on fairly light Hempstead clay loam on University Farm, St. Paul. Acid phosphate, at the rate of 480 pounds per acre, was added to three of these plats; sodium nitrate was added to three others at the rate of 360 pounds per acre; and three remained untreated as controls. Three varieties of wheat were sown, one on each of the square-rod plats to which the same fertilizer had been applied. These varieties were Glyndon Fife, C. I. 2873,[1] a hard red spring wheat, which is susceptible to stem rust in the field in Minnesota; Iumillo, C. I. 1736, a very resistant durum, and a moderately resistant selection from a durum-common cross. The seed was sown late and a heavy epidemic of stem rust developed.

As will be seen from Table II, the amount of stem rust which developed on the same variety in the different plats varied very slightly. On Glyndon Fife, the range was from 80 to 85 per cent; on Iumillo, it was from 15 to 20 per cent, and on the hybrid from 35 to 50 per cent. The lowest percentage of stem rust on Glyndon Fife was in the plat fertilized with acid phosphate, but on Iumillo and the hybrid the highest percentage was in the phosphate plats.

While the results are only indicative, it is quite clear that none of the fertilizers had an appreciable effect on the amount of stem rust. There was so little leaf rust that no data were obtained on its relative prevalence.

TABLE II.—*The percentages of stem rust on Glyndon Fife, Iumillo, and durum-common hybrid wheat in 1914, in the commercial fertilizer plats on University Farm, St. Paul, Minn.*

Kind and amount of fertilizer in pounds per acre.	Variety of wheat and percentage of stem rust.		
	Glyndon Fife.	Iumillo.	Semiresistant hybrid.
	Per cent.	*Per cent.*	*Per cent.*
Control, none	85	15+	35
Acid phosphate 480	80+	20—	50
Sodium nitrate 160	85	20—	35

[1] Accession number of the Office of Cereal Investigations, U. S. Department of Agriculture.

EXPERIMENTS IN THE GREENHOUSE IN 1914–15

As there was no observable effect of the different fertilizers on the amount of stem rust in the field during the summer of 1914, experiments were made in the greenhouse during the winter to determine whether an effect could be brought about under controlled conditions.

Wheat, oats, barley, and rye were sown in ordinary greenhouse soil, made up of sand, leaf mold, and black loam in 4-inch pots, where the plants were grown to maturity. Fertilizers were added to some and others were left as controls.

Wheat plants were inoculated with *Puccinia graminis tritici* and became quite uniformly infected with rust. The lightest infection was on plants which had been fertilized with acid phosphate. It was evident, however, that too much fertilizer had been used, since the plants were subnormal in vigor and size. Even when so much acid phosphate was used, however, that the plants were injured, the development of stem rust was not greatly inhibited.

Several pots of barley were fertilized with sodium nitrate and others with potassium chlorid. About 20 plants were inoculated in each series. Practically 100 per cent became infected. The uredinia, however, on the plants which had been fertilized with sodium nitrate were larger than were those on plants which had been fertilized with potassium chlorid. As there seemed to be some effect of fertilizers on the development of rust on plants growing in the greenhouse, an attempt was made to ascertain whether fertilizers would influence the development of stem rust on a semicongenial host.

Rye was inoculated with *Puccinia graminis tritici*, to which it is highly resistant. Some of the plants were grown in soil that had been heavily fertilized with sodium nitrate and others were grown in unfertilized soil. Two of the 13 plants which had been fertilized with nitrogen became infected, and one of the 19 grown in ordinary soil also developed uredinia. There were very few uredinia on any of the plants, but the individual uredinia were slightly larger on the plants which had been fertilized with sodium nitrate.

In order to get further data on the effects of fertilizers on the development of rust on a very highly resistant host, 58 barley plants growing in soil which had been heavily fertilized with sodium nitrate, and 40 growing in soil which had been heavily fertilized with potassium chlorid, were inoculated with *Puccinia graminis avenae*. Barley is almost immune from this form of stem rust although minute uredinia, usually smaller than a pinhead, sometimes are formed. One plant in the nitrate series became infected, and two of those in the potassium series developed uredinia. All of the uredinia were extremely small. The resistance of the barley plants to *Puccinia graminis avenae* certainly was not broken down by excessive fertilization with nitrogen, nor did the resistance seem to be increased by heavy fertilization with potassium chlorid.

In order to get still more information regarding the effect of stem rust on a congenial host, experiments were made with *Puccinia graminis avenae* on oats. Acid phosphate was added to the soil in some of the pots, while no fertilizer was added to others. So much acid phosphate was added that the tips of the leaves burned slightly. The degree of infection was about equal in both series. However, sometime after the plants had become infected, the uredinia seemed to be larger on the plants

which had not been fertilized with acid phosphate. This seemed to be quite natural, as the fertilized plants were small and unthrifty. They had been injured by excessive fertilization, but, even then, stem rust developed fairly well.

FIELD EXPERIMENTS WITH COMMERCIAL FERTILIZERS IN 1915

The field plats in 1915 were laid out on University Farm on rather heavy Hempstead loam soil [8] which had grown alfalfa for the preceding 15 years. Each plat was 1 square rod in extent and all were separated from each other by 3-foot alleys. The general arrangement is shown in Table III. The fertilizers were applied as indicated under "Experimental methods."

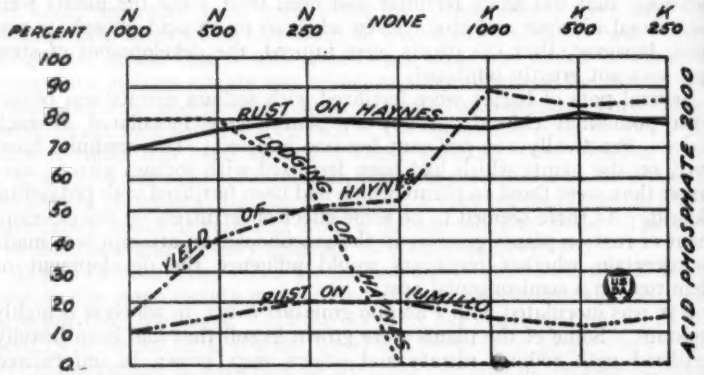

FIG. 2.—Graph showing the percentages of stem rust on Haynes Bluestem and on Iumillo and the percentage of lodging and acre yield in bushels (31 bushels=100 per cent) of Haynes Bluestem grown in plats which had received acid phosphate at the rate of 2,000 pounds per acre plus different amounts, in pounds, of sodium nitrate (N) and potassium chlorid (K) in the commercial fertilizer experiments in 1915 on University Farm, St. Paul, Minn.

———— rust on Haynes Bluestem.
– – – – – – – – lodging of Haynes Bluestem.
–·–·–·–·–·–·– yield of Haynes Bluestem.
–··–··–··–··–··– rust on Iumillo.

On May 6, Haynes Bluestem, C. I. 2874, was sown on half of each plat; and Iumillo, C. I. 1736, was sown on the other half. Haynes Bluestem is a hard red spring wheat, one of the best milling wheats grown in that region. But it is extremely susceptible to black stem rust, as is indicated by the fact that this variety has had an average infection of 70 per cent in the Minnesota rust nursery during the past eight years. Iumillo is a highly resistant durum on which the average percentage of rust in the Minnesota rust nursery from 1910 to 1920 was 10 per cent.

The seed was sown on May 6. Differences in the character of growth of the wheat in the different plats became apparent soon after the plants came up. The heaviest stand was on the nitrate plats, and the density of stand was in direct proportion to the amount of nitrogen applied. The plants were very succulent and soon began to lodge rather badly. The plants in the nitrogen plats also were a much darker green than those in the other plats. There was very little difference in the general

[8] See description of this soil under "Methods."

appearance of the plants in any of the plats except those which had been fertilized with nitrogen.

On July 1, stem rust began to appear on Haynes Bluestem. None had yet appeared on Iumillo. By July 27, Haynes Bluestem was uniformly infected with stem rust, but there were no apparent differences in the degree of infection in the different plats. On August 25, the plants were practically ripe and final observations were made. The results are given in Table III, which also is a diagram of the plats and their treatment. Figure 2 presents graphically the results from the series of seven plats receiving 2,000 pounds of acid phosphate and various other treatments.

TABLE III.—*The effect of commercial fertilizers on the percentages of stem rust on Iumillo and Haynes Bluestem wheats; and on the degree of lodging and yield of Haynes Bluestem in 1915 on University Farm, St. Paul, Minn.*

Infection, lodging, and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.						
	Kind.	Amount per acre.	Sodium nitrate.			None.	Potassium chlorid.		
			1,000	500	250		1,000	500	250
Per cent stem rust, Haynes Bluestem	Acid phosphate.	*Pounds.* 2,000	70	76	80	78+	77	82	78
Per cent stem rust, Iumillo			10	15	20	20—	15	12—	15
Per cent lodging, Haynes Bluestem			80	80	60	0	0	0	0
Bushels per acre, Haynes Bluestem			5	12. 8	15. 8	16. 6	27. 9	26. 2	26. 9
Per cent stem rust, Haynes Bluestem		1,000	76	78+	80	80+	85	86	85—
Per cent stem rust, Iumillo			20—	15	18	20+	20	20	20
Per cent lodging, Haynes Bluestem			60	60	60	40	60	60	40
Bushels per acre, Haynes Bluestem			8. 2	13. 9	14. 1	17. 8	22. 7	22. 9	26. 1
Per cent stem rust, Haynes Bluestem		500	83—	85+	82	83+	87	85—	88
Per cent stem rust, Iumillo			23	18	20	25—	15	15—	20—
Per cent lodging, Haynes Bluestem			40	40	40	20	0	20	0
Bushels per acre, Haynes Bluestem			6. 5	14. 3	17. 1	19. 1	19. 3	25. 5	31
Per cent stem rust, Haynes Bluestem		None.	80	82—	85+	83	81+	80+	86+
Per cent stem rust, Iumillo			22	22—	25—	25	18	15—	20
Per cent lodging, Haynes Bluestem			40	40	0	0	0	0	0
Bushels per acre, Haynes Bluestem			7. 8	16. 2	19. 8	22. 8	20. 7	21. 4	20. 5

It is quite evident from Table III that there is no definite correlation between the kind and amount of fertilizers applied and the amount of stem rust on the plants. The extreme range in percentage of stem rust is from 70 to 88 per cent. It so happens that the lowest percentage was in the plat which had received 1,000 pounds of sodium nitrate and 2,000 pounds of acid phosphate, while the highest percentage was in the plat which had received 500 pounds of acid phosphate and 250 pounds of potassium chlorid.[9] The heaviest infection, then, was in one of the

[9] The figures given always indicate the rate per acre at which the fertilizers were applied; for convenience the rate is given rather than the actual amount applied to each square-rod plat.

plats in which the plants theoretically should have been protected by the action of fertilizers. The percentage of infection in the plat which had received 1,000 pounds of sodium nitrate alone was 80, while in the one which had received 250 pounds it was 85. Certainly there seems to be no evidence that very heavy fertilization with nitrogen predisposed plants to stem rust, nor that acid phosphate and potassium chlorid protected them from it.

There are very striking differences, however, between the degree of infection on Haynes Bluestem and that on Iumillo. The average percentage of infection on Bluestem was approximately 80, while that on Iumillo was about 20. It is quite evident that the resistance of Iumillo was not broken down by excessive fertilization with sodium nitrate. In fact, there was more rust on the plants in the plats which had received no fertilizer whatever than there was on those in the plats which had received 1,000 pounds of sodium nitrate.

The results are particularly striking, as the soil on which the experiments were made was well supplied with nitrogen. It will be noted from Table III that the addition of nitrogen to the soil not only did not result in increased yields but actually caused a decrease, indicating quite clearly that the soil was not deficient in nitrogen. Any nitrogen which was added, therefore, was excessive.

While there was no correlation between kind and amount of fertilizers applied and the amount of stem rust which developed, there was a definite correlation between fertilization and lodging and yield. (See fig. 2.) The degree of lodging was much greater in the nitrogen plats than in any of the others and the yields were lower. The lowest yield, 5 bushels per acre, was in plat 1, which had received 2,000 pounds of acid phosphate and 1,000 pounds of sodium nitrate, and in which the degree of rust infection was lower than that in any of the other plats. The highest yield, 31 bushels per acre, by a rather remarkable coincidence, was in the plat in which the rust infection was 88 per cent, or higher than that in any of the other plats. It is a rather striking fact that the yield was always inversely proportional to the amount of nitrogen applied.

It is well known that wheat which has been overfertilized with nitrogen is likely to yield poorly. Not only does it often lodge badly but it frequently drys out, a phenomenon spoken of by farmers as "burning out." This burning out was quite conspicuous in the plats fertilized with nitrogen. It was supposed that there might be more rust on the plants which lodged badly in the nitrogen plats than on those which remained erect, but apparently such was not the case.

It is worthy of attention that the amount of rust on the plants is not necessarily an index of the damage which may be done. It seems quite likely that much of the injury usually attributed to rust on plants heavily fertilized with nitrogen probably is due to the direct effect of nitrogen rather than rust. For instance, wheat growing in soil which had received 1,000 pounds of sodium nitrate per acre had 80 per cent of rust infection and yielded only 7.8 bushels per acre, while that which had received 500 pounds of acid phosphate and 250 pounds of potassium chlorid had 88 per cent of rust but yielded 31 bushels per acre. It will be seen by referring to figure 1 (p. 347) that 88 per cent of infection is extremely heavy. The fact that plants so heavily infected with rust can produce well simply indicates that the visible amount of rust on the plants is no accurate measure of the damage done. It seems clear that

plants which have been fertilized properly can sometimes yield well even when heavily infected with stem rust.

There appeared to be a direct correlation, however, between the kind and amount of fertilizer applied and the percentage of infection by orange leaf rust, *Puccinia triticina* (Table IV). The percentages are not high because Haynes Bluestem is moderately resistant and Iumillo is very resistant in the field. The range in percentage of infection on Haynes Bluestem was from 10 to 40, the highest being in the nitrogen plats. The average percentage of infection in those plats which had received only nitrogen was 36; in those which had received nitrogen and phosphorus it was 29; in the phosphate plats, 22; in the potash plats, 17; in the phosphorus-plus-potassium plats, 21; while in the unfertilized control plat, it was 23. There was only a trace of leaf rust on Iumillo in all plats except the control and those which had received sodium nitrate. The highest percentage of infection was 10 in the plat receiving 1,000 pounds of nitrogen.

Evidently fertilization with sodium nitrate caused increased development of leaf rust, and phosphorus and potassium had some tendency to prevent its development, but it is quite likely that the effect may have been indirect. On the natural fertilizer plats there appeared to be no correlation between fertilization and development of leaf rust.

Unfortunately, very little leaf rust developed in any of the years subsequent to 1915, so additional data were not obtained and definite conclusions could not be drawn.

TABLE IV.—*The effect of commercial fertilizers on the degree of infection of Puccinia triticina in 1915 on Haynes Bluestem and Iumillo wheats in the plats on University Farm, St. Paul, Minn.*

Infection.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.						
	Kind.	Amount per acre.	Sodium nitrate.			None.	Potassium chlorid.		
			1,000	500	250		1,000	500	250
		Pounds.							
Percentage of leaf rust, Haynes Bluestem	Acid phosphate.	2,000	25	27	22	15	10	15	15
Percentage of leaf rust, Iumillo			5	2	1	1	1—	1—	1—
Percentage of leaf rust, Haynes Bluestem		1,000	30	30	25	25	15	20	20
Percentage of leaf rust, Iumillo			5	3	1—	1—	1	1—	1—
Percentage of leaf rust, Haynes Bluestem		500	40	35	30	25	12	15	15
Percentage of leaf rust, Iumillo			5	3	1	1	1—	1—	1—
Percentage of leaf rust, Haynes Bluestem		None.	40	40	27	23	20	15	15
Percentage of leaf rust, Iumillo			10+	5	7	3	1	1—	1—

EXPERIMENTS WITH COMMERCIAL FERTILIZERS PLUS NATURAL FERTILIZERS IN 1915

As previously outlined, the second method used in these experiments consisted in growing wheat on soil enriched with such natural fertilizers as barnyard manure, clover, and alfalfa, both alone and in various com-

binations with commercial fertilizers. The commercial fertilizers used were sodium nitrate, acid phosphate, and potassium chlorid. The soil was of the same type as that on which the experiments with commercial fertilizers alone were made. The plats which previously had grown cabbage and clover were contiguous to each other, while those on the alfalfa soil were a few rods distant.

The methods used in laying out the plats and applying the fertilizers were similar to those employed in the experiments with commercial fertilizers. The plats were 1 square rod in area and separated from each other by 3-foot alleys.

The commercial fertilizers were applied as indicated under the discussion of general methods.

Three series of plats were used. The soil in one series had grown cabbage the previous year, that in another had been in clover for several years, and that in the third had been in alfalfa for a like period. The ground was plowed, disked, and harrowed thoroughly before the seed was sown. Well-rotted barnyard manure, at the rate of 50 tons per acre, was applied to one plat in each series; 50 tons of manure, plus 500 pounds of sodium nitrate, per acre to one plat; 50 tons of manure, plus 1,000 pounds of acid phosphate, per acre to one plat; and 50 tons of manure, plus 1,000 pounds of potassium chlorid, per acre to one plat. The control plats received neither manure nor commercial fertilizers.

One-half of each plat was sown to Haynes Bluestem wheat, the other half to a hybrid (No. 4×942.10) originated in the Minnesota rust nursery. This hybrid was a cross between a durum (Kubanka, C. I. 2094) and Haynes Bluestem. It was a fixed type and averaged 30 per cent of rust in the Minnesota rust nursery in the five years from 1912 to 1917.

The disposition of the plats and the kind of soil and fertilizers used are shown in Table V. This table also gives the percentages of stem-rust infection for both varieties of wheat and the total number of days between the time Haynes Bluestem was sown and the time it ripened.

The stem-rust epidemic was assured by spraying with a suspension of spores such as had been used in the commercial fertilizer plats.

On July 1 the stand in the control plats on the alfalfa and clover soils was heavier than that in the control plat on the cabbage soil. The plants in all of the manured plats also were quite succulent and dark green in color. The commercial fertilizers apparently had influenced the character of growth but little up to this time.

TABLE V.—*The effect of commercial and natural fertilizers on the percentages of stem rust and leaf rust, and the total number of days for maturity for Haynes Bluestem and the resistant hybrid in 1915 on University Farm, St. Paul, Minn.*

Infection and growing period.	Fertilizer, kind and amount per acre.				
	50 tons of manure per acre, plus—				None.
	Nothing.	Sodium nitrate, 500 pounds.	Acid phosphate, 1,000 pounds.	Potassium chlorid, 1,000 pounds.	
CABBAGE SOIL					
Percentage of stem rust, Haynes Bluestem	87	88	85	90	70
Percentage of stem rust, hybrid	30	30	37	40	40
Percentage of leaf rust, Haynes Bluestem	7	15	10	12	15
Percentage of leaf rust, hybrid	27	27	25	30	20
Days for maturity of Haynes Bluestem	115	114	114	114	94
CLOVER SOIL					
Percentage of stem rust, Haynes Bluestem	85	80	86	87	70
Percentage of stem rust, hybrid	40	43	48	46	35
Percentage of leaf rust, Haynes Bluestem	12	15	12	12	20
Percentage of leaf rust, hybrid	30	27	25	20	28
Days for maturity of Haynes Bluestem	113	114	114	113	104
ALFALFA SOIL					
Percentage of stem rust, Haynes Bluestem	80	74	76	78	70
Percentage of stem rust, hybrid	38	38	35	36	25
Percentage of leaf rust, Haynes Bluestem	25	30	25	25	35
Percentage of leaf rust, hybrid	37	35	32	35	35
Days for maturity of Haynes Bluestem	114	115	115	115	110

On July 1 a few uredinia of stem rust were found on Haynes Bluestem but not on the hybrid. On July 27 Bluestem in all plats was quite uniformly infected with stem rust, although the percentage of infection on the plants in the cabbage soil was slightly higher than that on those in the clover soil, and those in the clover soil were slightly more severely affected than those on the alfalfa soil. The cause of these differences lay in the fact that the plats on the cabbage soil were nearest to heavily rusted barberry bushes. Therefore, a comparison between the amount of stem rust on plants in cabbage soil and those in alfalfa and clover soils can only be general. The degree of rust infection in these different series is correlated with the proximity of the plats to the rusted barberry bushes. On August 6 stem rust was spreading rapidly to stems and leaves on Bluestem in all the plats, but the spread on the hybrid was only moderate. The hybrid lodged severely in all of the manured plats, while the Bluestem lodged moderately in a few of them. Both varieties stood up perfectly in all of the control plats, except one in which the hybrid lodged somewhat.

From the data presented in Table V it at first appears that manure increased the susceptibility of the plants. But it is probable that this apparent increase of susceptibility was due partly at least to later maturity of the wheat, giving the rust a longer time to spread. The plants in the manured plats on the cabbage soil ripened 20 days after those in the control plats; those on the clover soil 10 days after those in the control plat; and those on the alfalfa soil 5 days after those in the control plat. There appears to be a complete correlation between the increase in percentage of stem rust on the Haynes Bluestem plants in the manured plats over that on those in the control plats, and the increased number of days required for maturity of the plants in the manured plats over those in the control plats. On the other hand, the percentage of infection in all of the control plats was 70, in spite of the fact that the plants in the different plats matured on different dates. The Bluestem in the control plat of the cabbage-soil series was mature on August 15,

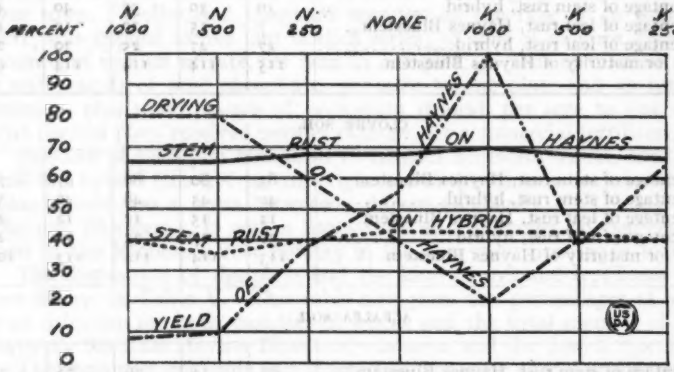

FIG. 3.—Graph showing the percentage of stem rust on Haynes Bluestem and on the hybrid and percentage of drying and acre yield in bushels (7.2 bushels=100 per cent) of Haynes Bluestem grown in plats which had received acid phosphate at the rate of 2,000 pounds per acre plus varying amounts in pounds of sodium nitrate (N) and potassium sulphate (K) in the commercial fertilizer experiments in 1916 on University Farm, St. Paul, Minn.

———— stem rust on Haynes Bluestem.
– – – – – – stem rust on hybrid.
–·–·–·–·– drying of Haynes Bluestem.
–·–·–·–·–·– yield of Haynes Bluestem.

in the control plat of the clover-soil series on August 22, and in the control plat of the alfalfa-soil series on August 28. Up to August 28, therefore, the dates of maturity seemed to have no effect on the amount of rust. After this date, however, rust again spread rather rapidly on those plants which remained green, but it is impossible to say just how much effect delayed maturity had on the percentage of rust in this series.

There is no definite correlation between fertilization and the percentage of infection of *P. triticina*, which is in sharp contrast with the results obtained on the commercial fertilizer plats. The hybrid appeared more susceptible than Haynes Bluestem, but the highest percentage of infection, 35 for both varieties, was on unfertilized alfalfa soil.

EXPERIMENTS WITH COMMERCIAL FERTILIZERS IN 1916

The commercial fertilizer plats in 1916 were the same plats actually used in 1915. A resistant hybrid wheat was used instead of Iumillo, however, and potassium sulphate was used instead of potassium chlorid.

The size and position of the plats and the rate of application of fertilizers were exactly the same as in 1915. (See Table VI.)

The hybrid (No. 5 × 2223), used in place of Iumillo, was a cross between a durum, Kubanka, C. I. No. 2094, and a common hard red spring variety, Preston, C. I. 3328. It was a fixed type, in the F_8 generation, originated by Freeman and Johnson. The average amount of stem rust on the hybrid in the Minnesota rust nursery from 1912 to 1916 was 30 per cent. It is slightly more susceptible, therefore, than Iumillo.

Both varieties were sown on May 13, which is rather late for the district. However, the spring was late. The temperatures were abnormally low during April, May, and June, while the precipitation was abnormally high. During July and August, however, the conditions were reversed. These abnormal climatic conditions undoubtedly affected the development of stem rust, the date of maturity of the plants, and the yield. The results obtained, as well as the plan of the experiment, are shown in Table VI. Figure 3 shows the results in the seven plats of the series receiving 2,000 pounds of acid phosphate.

TABLE VI.—*The disposition of the commercial fertilizer plats and the percentages of stem-rust infection on Haynes Bluestem wheat and the hybrid, and the degree of drying and yield in bushels per acre of Haynes Bluestem in 1916 on commercial fertilizer plats on University Farm, St. Paul, Minn.*

Infection, drying, and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.						
	Kind.	Amount per acre.	Sodium nitrate.			None.	Potassium sulphate.		
			1,000	500	250		1,000	500	250
Percentage of stem rust, Haynes Blustem	Acid phosphate.	*Pounds.* 2,000	65.0	65.0	68.0	68.0	70.0	68.0	67.0
Percentage of stem rust, hybrid			40	36	40	43	43	43	40
Percentage of drying, Haynes Bluestem			80	80	60	40	20	40	40
Bushels per acre, Haynes Bluestem			0.6	0.7	2.7	4	7.2	2.8	4.7
Bushels per acre, hybrid			6.6	6.9	7.8	14.3	18.8	18.6	16.8
Percentage of stem rust, Haynes Bluestem		1,000	65	66+	66+	66	70	70	70
Percentage of stem rust, hybrid			40−	38	40	42	43+	43	43
Percentage of drying, Haynes Bluestem			80	80	60	40	40	40	40
Bushels per acre, Haynes Bluestem			0.6	0.5	1.0	1.3	4.6	1.7	3.5
Bushels per acre, hybrid			5.2	6.9	7.5	13.7	18.6	17.4	17.9
Percentage of stem rust, Haynes Bluestem		500	66	66+	67	69	70	70+	70+
Percentage of stem rust, hybrid			45	41+	42	43+	45	45	45
Percentage of drying, Haynes Bluestem			60	80	80	40	40	40	40
Bushels per acre, Haynes Bluestem			0.3	0.5	0.7	1.4	2.7	1.7	2.8
Bushels per acre, hybrid			7.4	9.6	9.1	14.6	20.3	13.0	19.4
Percentage of stem rust, Haynes Bluestem		None.	67+	65	67+	70	71	70	70+
Percentage of stem rust, hybrid			43	42	42−	43+	45	43+	44
Percentage of drying, Haynes Bluestem			80	60	60	40	40	40	40
Bushels per acre, Haynes Bluestem			0.5	0.5	0.7	1.8	2.2	1.4	2.8
Bushels per acre, hybrid			7.7	10.0	8.1	16.1	16.8	11.2	16.7

Stem rust first began to appear on June 16, after which it developed and spread rapidly and uniformly until checked by the hot, dry weather. The growth of the wheat plants also was checked by the drought, the plants in the plats treated with nitrogen plus acid phosphate being injured most severely. As there was some drying and premature ripening, the rust did not become as abundant as it had in 1915. It will be noted that in 1916 the period between sowing and harvesting was only 85 days, while in 1915 it was 116 days. The range in percentage of stem-rust infection on Haynes Bluestem in 1916 was from 65 to 70 per cent and on the resistant hybrid it was from 36 to 45 per cent. The narrow range may have been due partly to the fact that the plants ripened prematurely. It is interesting to note that, as in 1915, the highest percentage of stem rust on Haynes Bluestem was not in the nitrate plats. In fact, the average percentage of infection was lower in those plats which had been fertilized with nitrogen alone than in the control plat and in those which had received acid phosphate and potassium sulphate. The average percentage of stem rust also was slightly higher on the hybrid in the phosphate and potassium than in the nitrogen plats. It is perfectly clear, therefore, that excessive fertilization with nitrogen failed entirely to predispose either the susceptible Haynes Bluestem or the resistant hybrid to stem rust; nor did phosphorus and potassium protect them. Whatever slight effect there may have been appeared to be just the opposite of what one would expect. There was a very definite lack of correlation between fertilization and the development of stem rust (see Table VI and fig. 3), a fact for which weather conditions probably were partly responsible.

About a week after the plants had headed, the weather became very hot and dry. The growth of the plants, especially in the nitrate and phosphate plats, was severely checked and many of them began to dry up and crinkle. The crinkling was fairly uniform and could not be attributed entirely to the effect of fertilizers, although it was consistently worse in the nitrate plats than in any others. As would be expected, "burning out," due to heavy fertilization with nitrogen, is aggravated by hot dry weather. As "burning out" really means premature ripening, nitrogen actually may cause a decrease in the amount of rust in years when the weather is hot and dry. But in years when the growing conditions are favorable it may increase rust infection by prolonging the growing period. The degree of shriveling of seed was in direct proportion to the amount of drying, and the yield, in general, was inversely proportional to the amount of shriveling. For instance, the acre yield of Haynes Bluestem was less than 1 bushel in all of the plats in which the degree of drying was 80, while on all of those in which the drying was less than 60, the yield was over 1 bushel per acre.

The yields in 1916 were deplorably low, of course, as they were generally throughout the hard red spring wheat region. For instance, in 1915, the average acre yield in Minnesota was 17 bushels, in South Dakota 17, and in North Dakota 18.2 bushels. In 1916, they were 7.5, 6.3, and 5.5 bushels, respectively (*35, p. 593*). This was attributed principally to the terrific stem-rust epidemic, the most destructive since 1904. It is interesting, however, to note that the average percentage of stem rust on all plats in 1915 was considerably higher than it was in 1916. But the yields in 1915 were very much higher than they were in 1916. In 1915 the lowest yield in the commercial fertilizer plats was 5 bushels

per acre and the highest was 31 bushels. In 1916 the lowest yield was only 0.3 of a bushel per acre and the highest only 7.1 bushels. Either the drought alone caused much of the damage in 1916, or the drought aggravated the rust damage. As Weaver (*40*) has shown that the rate of transpiration of rusted cereal plants is considerably greater than that of rust-free plants, it seems probable either that the loss of water or the water requirement is an important factor in rust damage. This would explain why plants fertilized with nitrogen apparently or actually are injured more by rust than are other plants. The susceptibility of the plants to rust is not necessarily increased by the nitrogen, but the rust is especially dangeous to such plants, because it increases the water requirement which already is too high for safety. That the great damage to the Haynes Bluestem in the commercial fertilizer plats was caused by a combination of drought and stem rust, and not by drought alone, is suggested by the fact that the resistant hybrid yielded from two to about twelve times as much as the Bluestem. (See Table VI.) It is possible, however, that the hybrid may be somewhat drought resistant. While it is difficult to differentiate clearly between drought injury and rust injury, it is quite evident that the degree of rust infection is not necessarily an index of the amount of injury it will cause.

The results obtained in 1915 and 1916 indicate clearly that wheat may yield well in spite of heavy stem-rust infection, if weather conditions are favorable to the crop. On the contrary, a moderate rust attack either causes or appears to cause severe damage when weather and soil conditions are unfavorable to the wheat. It is probable also that the effect of fertilizers may be different under different climatic conditions. In a hot, dry year, wheat fertilized with nitrogen may "burn out," thus checking the spread of stem rust; while in a cool, moist season, maturity may be delayed by nitrogenous fertilizers, thus giving the rust more time in which to develop.

EXPERIMENTS WITH COMMERCIAL FERTILIZERS PLUS NATURAL FERTILIZER IN 1916

The general plan of the plats treated with commercial plus natural fertilizer is shown in Table VII. The plats were on heavy Hempstead clay loam on University Farm, which had grown alfalfa for several years previously. Haynes Bluestem and the same hybrid wheat which was used in 1915 also were used in this experiment. Each variety occupied 1 full square rod instead of half that area as in 1915. The general conditions of the experiment were the same as those for the commercial fertilizer experiments in 1916.

TABLE VII.—*The disposition of the plats treated with commercial plus natural fertilizer and the percentages of rust on Haynes Bluestem and the hybrid in the plats on University Farm, St. Paul, Minn., in 1916*

Infection.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.			
	Kind.	Amount per acre.	Sodium nitrate. 500.	Potassium sulphate 1,000.	None.	Dried blood 650.
Percentage of stem rust, Haynes Bluestem.	Manure.	10 tons...	58	60	61	63
Percentage of stem rust, hybrid			40	41	43	42
Percentage of stem rust, Haynes Bluestem.		25 tons...	58	58	61	63
Percentage of stem rust, hybrid			40	40	43	42
Percentage of stem rust, Haynes Bluestem.		Control..	55	.....	.....	.....
Percentage of stem rust, hybrid			38	.....	.....	.....

The plants on the manured plats were uniform and were much more vigorous and darker green in color than those on the control plat. One week after heading out, the plants were injured by drought. The peduncles of the plants broke over or crinkled at the point where they emerged from the sheath. This occurred so uniformly on all the plats that it could not be attributed entirely to the effect of the fertilizers. The same effect was noted on the plats in the experiment with the commercial fertilizers for this same year.

The plants lodged badly on all the manured plats, while there was no lodging in the control plat. The average percentage of lodging in the plats receiving 10 tons of manure per acre was 40, while in those receiving 25 tons it was 60 per cent.

The average percentages of stem rust are shown in Table VIII. The range on Haynes Bluestem was from 55 to 63 per cent, and on the hybrid from 38 to 43 per cent. The differences were so small that one would be fully justified in concluding that the effect of the fertilizers on the susceptibility of the host to attacks by stem rust is negligible.

The yields were greatly reduced by the drought and the rust. There was a sharp difference in the ability of the two varieties to withstand the effects of the drought, a difference due partly at least to slightly earlier maturity of the hybrid. The average yield of Haynes Bluestem was less than a bushel per acre, while that of the hybrid was between 5 and 6 bushels per acre.

TABLE VIII.—*Summarized data on the effect of barnyard manure on the percentage of stem rust on Haynes Bluestem and on the hybrid in the plats treated with commercial plus natural fertilizer in 1916 on University Farm, St. Paul, Minn.*

Plats.	Number of plats included.	Average percentage of rust.		Range of rust percentage.	
		Bluestem.	Hybrid.	Bluestem.	Hybrid.
All	9	58.5	40.2	55–63	34–43
Control	1	55	38	55	38
Receiving 10 tons manure per acre	4	60.5	41.5	58–63	40–43
Receiving 25 tons manure per acre	4	60	41.2	58–63	40–43

EXPERIMENTS WITH COMMERCIAL FERTILIZERS IN 1917

General Plan

In 1917, experiments were made on three different farms. The general plan was the same for each, but there were some minor variations. The series were as follows:

FIRST SERIES, UNIVERSITY FARM.—The plats were laid out in exactly the same place as in the previous two years. The soil therefore was the heavy Hempstead clay loam.

SECOND SERIES, QUINN FARM.—The plats were laid out on light Hempstead clay loam. They were about 1½ miles from those in the first series. The soil on the Quinn farm, while of the same general type as that on University farm, was much lighter, contained more sand and was not so well supplied with nitrogen.

THIRD SERIES, ANOKA.—The plats were laid out on Merrimac loamy sand near Anoka. The soil was very light and deficient in nitrogen.

Results of First Series—University Farm

The experiments on University Farm were made on the same plats used in the previous two years; but acid phosphate and potassium were not applied, because it was desired to ascertain whether there would be any residual effect of these fertilizers on the plants themselves and on their resistance to stem rust. Sodium nitrate was applied as usual, as the residual effect is negligible. On April 28 one-half of each plat was sown to Marquis, C. I. 3641, which is very susceptible to stem rust in Minnesota, and the other half was sown to the same hybrid which was used in 1916.

The weather conditions in 1917 were quite different from those in 1916. The spring of 1916 was wet and cold, but soon after the plants headed it became very hot and dry. In 1917, the growing conditions generally were favorable for wheat. This is indicated clearly by the fact that there was practically no premature ripening and the yields were good in practically all of the plats. Stem rust did very little damage throughout the hard red spring wheat region and the yield of wheat was fairly satisfactory, except in North Dakota where drought reduced it. The average yield in Minnesota was 17.5 bushels per acre, in North Dakota 8 bushels per acre, and in South Dakota 14 bushels per acre (*35*).

The plan of the experiment and the results obtained, including the percentages of stem-rust infection on both varieties in each plat, are

given in Table IX. At first, it appears that there is a definite correlation between the amount of stem rust and the kind of fertilizer applied. The highest average percentage of rust on Marquis was on the plats which had received sodium nitrate at the rate of 1,000 pounds per acre, the next highest on those which had received 500 pounds, and the third highest, on those which had received 250 pounds. There appears, therefore, to be a direct correlation. The higher percentage of rust in these plats was due partly, at least, to delayed maturity, as the plants matured a week or 10 days later than did those in the other plats. The density of stand also no doubt made it possible for the rust to develop more abundantly, as the plants lodged badly and moisture was retained much longer than on those plants which did not lodge. (See Pl. 1, 2, and 3.)

TABLE IX.—*The disposition of the commercial fertilizers and the percentages of stem rust on Marquis and the hybrid, and the percentages of yellow-berry, seed shriveling, lodging, and the acre yields of Marquis in 1917 in the plats on University Farm, St. Paul, Minn.*

Infection, yellow-berry, seed shriveling, lodging, and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.						
	Kind.	Amount per acre.	Sodium nitrate.			None.	Potassium sulphate residual[a]		
			1,000	500	250		1,000	500	250
		Pounds.							
Percentage of stem rust, Marquis	Acid phosphate residual[a]	2,000	55	33	31	33	28	29	28
Percentage of stem rust, hybrid			17	8	7	6	7	4	2
Percentage of yellow-berry, Marquis			0	0	50	50	80	90	99
Percentage of seed shriveling, Marquis			70	70	15	0	0	0	0
Percentage of lodging, Marquis			100	80	60	0	0	0	0
Bushels per acre, Marquis			12.8	14.1	28.2	35.3	24.3	21.7	22.0
Percentage of stem rust, Marquis		1,000	60	37	35	33	32	31	26
Percentage of stem rust, hybrid			11	19	6	6	7	6	5
Percentage of yellow-berry, Marquis			0	0	10	75	70	80	80
Percentage of seed shriveling, Marquis			70	60	15	0	0	0	15
Percentage of lodging, Marquis			100	90	40	20	0	0	0
Bushels per acre, Marquis			15.8	17.5	31.8	30.5	26.9	25.9	23.9
Percentage of stem rust, Marquis		500	65	37	37	32	34	31	27
Percentage of stem rust, hybrid			10	10	5	6	6	8	2
Percentage of yellow-berry, Marquis			0	0	5	40	70	60	90
Percentage of seed shriveling, Marquis			30	30	20	0	0	0	0
Percentage of lodging, Marquis			100	90	80	30	0	20	0
Bushels per acre, Marquis			18.6	20.7	26.1	32.4	30.8	35.7	32.6
Percentage of stem rust, Marquis		None.	67	35	38	28	32	31	25
Percentage of stem rust, hybrid			9	8	4	5	7	6	1
Percentage of yellow-berry, Marquis			0	0	5	40	60	75	70
Percentage of seed shriveling, Marquis			40	30	15	15	0	0	0
Percentage of lodging, Marquis			80	80	60	0	0	0	0
Bushels per acre, Marquis			14.7	22.0	25.8	40.8	33.2	32.5	29.6

[a] Fertilizers applied, at rate indicated, in 1916, but not in 1917.

It will be noted that the effect of nitrogen in a year like 1917 may be quite different from that in a year like 1916. In 1916 the weather became very hot and dry shortly after the plants headed. The plants in the nitrate plats suffered more from the drought than did those in any of the other plats. The result, of course, was that the development of the rust was checked by the premature ripening of the plants. In 1917, on the other hand, the growing conditions were good, there was but little real burning out the stand in the nitrogen plats was very heavy, the individual plants were succulent, and maturity was greatly delayed, thus making conditions especially favorable for the development of stem rust.

The date of maturity often has a great influence on the degree of rust infection. It is a very commonly observed and well-known fact that early maturing varieties often escape rust, while late maturing ones may be injured severely. In certain years the rust epidemic appears just after the earlier-maturing variety has ripened, but early enough to attack the late variety while it is still susceptible. The plants in the plats heavily fertilized with nitrogen in 1917 matured several days later than those in the other plats, and the opportunity for infection was thus increased. The rust percentage on a given date was uniform in all of the plats, but the weather was still favorable for the development of rust after the earlier-maturing plants were harvested and while the later-maturing plants still were green. Naturally more rust developed on the latter. This is illustrated in Table XII with data taken from the plats on the Quinn farm.

The residual effect of the potassium and phosphate fertilizers applied to the plats prior to 1917 apparently had little or no effect on the susceptibility or resistance of the plants to stem rust. (Table IX.) The percentages of rust in the plats which had received only acid phosphate the previous year actually were slightly higher than those in the control plat. There clearly was no protective action in the potassium plats, nor in those which had received both acid phosphate and potassium sulphate. It was possible to note the residual effect of the fertilizers applied in 1916 only on one character, that of yellow-berry, which was very high in some of the plats which had received no nitrogen. The results from the seven plats receiving 2,000 pounds of acid phosphate are illustrated graphically in figure 4.

It will be seen from Table X that there was a great increase in the amount of straw on the nitrogen plats. In some cases it is almost double that on certain potassium plats. Just the reverse is true of the total weight of seed per plat. The average yield of grain for all plats receiving applications of nitrogen was 20.7 bushels per acre, while the average yield for all those not receiving nitrogen was 29.9 bushels per acre, a difference of 9.2 bushels in favor of the plats not receiving nitrogen. The ratio of weight of seed to weight of straw shows clearly the effect of the fertilizers applied to the soil on the growth of the plant. Likewise, the yields from the nitrogen plats is inversely proportional to the amount of nitrogen applied.

There appears to be little correlation between rust percentage and yield. This is especially clear when one compares the data for the plats which received 500 pounds of sodium nitrate with those for the plats which received 250 pounds (Table IX). The highest yield was obtained on the untreated control. The ratio of seed to straw for all plats receiving applications of nitrogen is 1:4.6; while the ratio for all plats not receiving applications of nitrogen is 1:2.5. The nitrogen clearly

was detrimental, but it was because it promoted excessive production of straw and low yield of grain rather than because of any effect it may have had on the rust. The highest percentage of rust was 67 in the plat which had received only sodium nitrate at the rate of 1,000 pounds during each of the three years. But this amount of rust does not necessarily cause a decrease in yield, as is shown by the fact that in 1915 the highest yield in the commercial fertilizer plats was 31 bushels per acre from a plat in which the percentage of stem rust was 88 per cent.

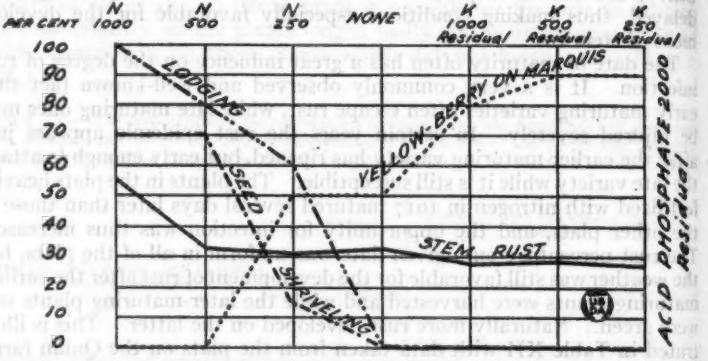

FIG. 4.—Graph showing the percentages of stem rust, yellow-berry, seed shriveling, and lodging of Marquis wheat grown in plats which had received acid phosphate at the rate of 2,000 pounds per acre and different amounts, in pounds, of potassium sulphate (K) in 1916 and different amounts of sodium nitrate (N) in 1917, in commercial fertilizer experiments in 1917 on University Farm, St. Paul, Minn.

——————— stem rust.
- yellow-berry.
-.- seed shriveling.
-..-..-..-..-..-..-..-..-..-..-..-..-..-.. lodging.

TABLE X.—*The effect of fertilizers on the yields, in kilograms, of seed and straw of Marquis wheat and on the ratio of seed to straw in the commercial fertilizer plats in 1917 on University Farm, St. Paul, Minn.*

Weight of seed and straw, and ratio of seed to straw.	Fertilizer. Kind.	Fertilizer. Amount per acre.	Fertilizer, kind and amount (pounds) per acre. Sodium nitrate. 1,000	Sodium nitrate. 500	Sodium nitrate. 250	None.	Potassium chlorid residual.[a] 1,000	Potassium chlorid residual.[a] 500	Potassium chlorid residual.[a] 250
		Pounds.							
Weight of seed	Acid phosphate residual.[a]	2,000	1.1	1.3	2.5	3.2	2.2	1.9	2.0
Weight of straw			7.7	8.4	7.9	7.4	5.3	5.6	4.7
Ratio, seed to straw			1:7	1:6.4	1:3.2	1:2.3	1:2.4	1:3	1:2.4
Weight of seed		1,000	1.4	1.6	2.8	2.8	2.4	2.3	2.1
Weight of straw			8.4	7.0	7.8	7.5	6.0	5.3	4.6
Ratio, seed to straw			1:6	1:4.4	1:2.8	1:2.7	1:2.5	1:2.3	1:2.2
Weight of seed		500	1.7	1.9	2.3	2.9	2.8	3.2	2.9
Weight of straw			9.1	7.7	9.2	9.5	7.6	7.5	7.3
Ratio, seed to straw			1:5.4	1:4.1	1:4	1:3.3	1:2.7	1:2.3	1:2.5
Weight of seed		None.	1.3	2.0	2.3	3.6	3.0	2.9	2.6
Weight of straw			8.7	7.8	8.0	7.5	6.6	7.1	7.1
Ratio, seed to straw			1:6.7	1:3.9	1:3.5	1:2.1	1:2.2	1:2.4	1:2.7

[a] Fertilizers applied, at rate indicated, in 1916, but not in 1917.

Results from Second Series—Quinn Farm

The second series was laid out on rather light, Hempstead clay-loam soil and consisted of 32 plats. The general plan was similar to that followed on University Farm, except that on the Quinn farm certain combinations were made between sodium nitrate and potassium chlorid, and other combinations of sodium nitrate, potassium chlorid and acid phosphate. The plats were sown to Marquis and the hybrid, as in the first series. The disposition of the plats, the rate and kind of fertilizer applied to each, the percentage of stem rust on both varieties, and the yield for Marquis are given in Table XI.

Table XI.—*The disposition of the fertilizers applied, the percentages of stem rust on Marquis and the hybrid, and the yields in bushels per acre of Marquis in 1917 in the commercial fertilizer plats on the Quinn farm, St. Paul, Minn.*

Infection and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.							
	Kind.	Amount per acre.	Sodium nitrate.			None.			Sodium nitrate.	
			1,000	500	250				500	500
		Pounds.								
Percentage of stem rust, Marquis	Acid phosphate.	2,000	80	28	12	5	6—	5—	10	9
Percentage of stem rust, hybrid.			20	10	2	1	2	1	1	1
Bushels per acre, Marquis.....			24.2	43.9	27.6	14.3	25.1	22.3	20.2	20.0
Percentage of stem rust, Marquis		1,000	76	65	9	8	8	8	12	11
Percentage of stem rust, hybrid.			20	8	3	1	1	1	2	2
Bushels per acre, Marquis.....			33.4	29.6	33.6	30.2	30.0	31.7	42.9	38.1
Percentage of stem rust, Marquis		500	10	9	8	7	7	7	10	11
Percentage of stem rust, hybrid.			3	1	1	1	1	1	5	3
Bushels per acre, Marquis.....			42.7	37.8	29.0	21.9	23.6	23.2	40.5	39.6
Percentage of stem rust, Marquis		None.	11	11	10	10	10	10	27	18
Percentage of stem rust, hybrid.			4	1	1	1	1	2	20	22
Bushels per acre, Marquis.....			33.7	42.7	37.4	29.0	30.1	28.4	37.6	16.4
							500	250	500	250
							Potassium chlorid.			
			Fertilizer, kind and amount (pounds) per acre.							

It was pointed out in the discussion of results on University Farm that the increased percentage of rust on a few of the plats to which sodium nitrate had been applied was due to delayed maturity and not to an increased degree of susceptibility. This fact is illustrated clearly in Table XII. Even with the slight difference in these plats, it is apparent that in these experiments the effect of the fertilizers on the susceptibility of the wheat to attacks by stem rust is too small to be significant.

The percentages of stem rust for the various plats in the column headed "Percentage of rust, August 10," it will be noted, are very uniform. For the hybrid they run, 1, 2, 2, 5, 2, 4, and 1 per cent, respectively, for plats 22, 30, 23, 31, 24, 32, and 28, respectively. This same series ten days

later, however, as shown in column headed "Percentage of rust August 20," is far from uniform. For the hybrid, they run 1, 2, 5, 20, 3, 22, and 1 per cent, respectively, for the same plats. When compared with the column giving the number of days from planting to maturity, it will be noted that there is a direct correlation between the dates of maturity and the percentages of stem rust. It will be noted further that it was only on the plats fertilized with sodium nitrate that there was sufficient delay in maturity to result in a higher percentage of stem rust.

TABLE XII.—*The effect of fertilizers on the date of maturity and on the percentages of stem rust on Marquis and the hybrid in 1917 on the commercial fertilizer plats on the Quinn farm, St. Paul, Minn.*

Plat No.	Kind and amount of fertilizer [a] in pounds per acre.	Number of days from planting to maturity.		Percentage of stem rust.			
				Aug. 10 (100 days after sowing).		Aug. 20 (110 days after sowing).	
		Marquis.	Hybrid.	Marquis.	Hybrid.	Marquis.	Hybrid.
22	K 250. P 500.	100	101	7	1	7	1
30	K 250.	102	103	10	2	10	2
23	K 500. N 500. P 500.	104	105	9	2	10+	5+
31	K 500. N 500.	108	110	14	5	27	20
24	K 250. N 500. P 500.	104	105	9	2	11	3+
32	K 250. N 500.	108	111	12	4	18	22+
28	Control.	102	104	10	1	10	1

[a] N=sodium nitrate; K=potassium chlorid; P=acid phosphate.

The percentages of stem rust are high in four of the plats in the upper left-hand corner of Table XI. Unfortunately, these plats were in the shadow of a barn during part of the day, so the plants remained wet longer after dews and rains than did those in any of the other plats. It was clearly evident that this shading and late maturity were chiefly responsible for the increased percentage of rust in this part of the field.

The fertilizers affected the general growth of these plants more than those on University Farm. The average height of the plants in all plats which received applications of sodium nitrate was 40.3 inches, while the average height of the plants in all other plats was 36.25 inches. These data, with others on weight of seed and of straw, and the ratio of seed to straw, are given in Table XIII.

TABLE XIII.—*The effect of fertilizers on the height of plants in inches; on the yield, in kilograms, of seed and straw; and on the ratio of seed to straw, of Marquis in 1917 in the commercial fertilizer plats on the Quinn farm, St. Paul, Minn.*

Straw height, seed and straw weight, and ratio of seed to straw.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.								
	Kind.	Amount per acre.	Sodium nitrate.			None.				Sodium nitrate.	
			1,000	500	250					500	500
		Pounds.									
Height of straw	Acid phosphate.	2,000	40	44	44	35	36	32	42	40	
Weight of seed			2.2	3.9	2.5	1.3	2.2	2.0	3.6	3.6	
Weight of straw			12.8	13.7	11.0	6.5	6.5	5.3	11.7	11.5	
Ratio, seed to straw			1:5.8	1:3.5	1:4.4	1:5	1:3	1:2.7	1:3.3	1:3.2	
Height of straw		1,000	39	39	45	39	39	38	42	41	
Weight of seed			3.0	2.6	3.0	2.7	2.7	2.8	3.9	3.4	
Weight of straw			9.9	13.8	12.4	8.1	8.4	8.4	12.8	12.4	
Ratio, seed to straw			1:3.3	1:5.3	1:4.1	1:3	1:3.1	1:3	1:3.3	1:3.6	
Height of straw		500	40	40	36	35	35	33	36	38	
Weight of seed			3.8	3.4	2.6	1.9	2.1	2.1	3.6	3.5	
Weight of straw			12.1	10.3	8.4	5.6	6.2	5.9	11.1	11.2	
Ratio, seed to straw			1:3.2	1:3	1:3.2	1:2.9	1:3	1:2.8	1:3.1	1:3.2	
Height of straw		None.	38	39	40	38	38	37	37	37	
Weight of seed			3.0	3.8	3.3	2.6	2.7	2.5	3.4	1.5	
Weight of straw			10.8	11.5	10.5	7.3	7.4	7.1	11.1	11.2	
Ratio, seed to straw			1:3.6	1:3	1:3.2	1:2.8	1:2.7	1:2.8	1:3.2	1:7.5	
							500	250	500	250	
							Potassium chlorid.				
			Fertilizer, kind and amount (pounds) per acre.								

The plants in the plats fertilized with sodium nitrate tillered better, were more vigorous, and yielded better than those on the plats which had received no nitrogen. The average yield from the nitrate plats was 35.8 bushels per acre, while that from those receiving no nitrogen was 25.8 bushels, a difference of ten bushels in favor of the plats fertilized with nitrogen. The ratios of seed to straw were quite uniform. The effect of sodium nitrate on yield was quite different from that in the plats on University Farm. Here there was a difference of 9.2 bushels in favor of the plats not receiving nitrogen, while on the Quinn farm there was a difference of 10 bushels in favor of the plats receiving nitrogen. Evidently the soil on the Quinn farm was deficient in available nitrogen, while that on University Farm was not.

The data in Table XIV show also the correlation between the fertilizers applied and the percentages of "yellow-berry" and protein in the seed. The average percentages of yellow-berry and protein content of the seed for all plats which received applications of sodium nitrate are 14.5 per cent and 14.7 per cent, respectively, compared with 67 per cent and 12.8 per cent, respectively, as the averages for all plats which received no

applications of sodium nitrate.[10] Heavy applications of nitrogen caused some lodging and shriveling of the seed.

TABLE XIV.—*The effect of fertilizers on the percentages of yellow-berry, protein in seed, seed shriveling, and lodging of Marquis in 1917 in the commercial fertilizer plats on the Quinn farm, St. Paul, Minn.*

Yellow-berry, protein in seed, seed shriveling, and lodging.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.							
	Kind.	Amount per acre.	Sodium nitrate.			None.			Sodium nitrate.	
			1,000	500	250				500	500
		Pounds.								
Percentage of yellow-berry	Acid phosphate.	2,000	0	20	80	70	85	85	25	10
Percentage of protein in seed[a]			16.6	15.1	13.8	12.9	13.3	13.6	14.3	14.4
Percentage of seed shriveling			30	0	0	0	0	0	0	20
Percentage of lodging			80	30	0	0	0	0	20	20
Percentage of yellow-berry		1,000	1	5	45	80	85	70	5	1
Percentage of protein in seed[a]			16.4	15.9	14.5	12.6	11.7	12.7	14.9	15.5
Percentage of seed shriveling			30	20	0	20	0	0	20	20
Percentage of lodging			40	70	20	0	0	0	30	40
Percentage of yellow-berry		500	0	10	50	50	70	60	15	8
Percentage of protein in seed[a]			16.3	14.5	13.4	13.2	12.4	12.6	13.6	14.5
Percentage of seed shriveling			0	0	0	0	0	0	20	20
Percentage of lodging			30	0	0	0	0	0	20	20
Percentage of yellow-berry		None.	0	2	5	50	50	50	2	5
Percentage of protein in seed[a]			15.9	14.8	14.0	13.1	12.4	13.1	13.9	14.0
Percentage of seed shriveling			30	20	0	0	0	0	20	0
Percentage of lodging			50	0	0	0	0	0	30	20
							500	250	500	250
							Potassium chlorid.			
			Fertilizer, kind and amount (pounds) per acre.							

[a] On dry basis.

From the data obtained, it is very evident that the normal physiology of the plants was changed considerably on the different plats. This is shown both by observations on the morphology of the plants and by chemical analysis, but these changes apparently affected stem rust only indirectly by affecting density of stand and time of ripening.

RESULTS FROM THIRD SERIES—ANOKA

The third series was laid out on a light, loamy sand near Anoka, Minn. The plan of the plats was similar to that of the second series, both in disposition of the plats and in the application of fertilizers. The full square rod was sown to the one variety, Marquis, instead of two varieties as in the preceding experiments. The disposition of the plats, the rate and

[10] These analyses were made by Dr. C. H. Bailey, in charge of the section of cereal technology of the division of agricultural biochemistry of the University of Minnesota.

kind of fertilizer applied to each, the percentages of stem rust, yellow-berry and seed shriveling, and the yield in bushels per acre are given in Table XV.

TABLE XV.—*The disposition of the commercial fertilizer plats and the effect of the fertilizers on the percentages of stem rust, yellow-berry and seed shriveling, and on yield in bushels per acre for Marquis wheat, at Anoka, Minn., in 1917*

Infection, yellow-berry, seed shriveling and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.							
	Kind.	Amount per acre.	Sodium nitrate.			None.			Sodium nitrate.	
			1,000	500	250				500	500
		Pounds.								
Percentage of stem rust	Acid phosphate.	2,000	15	11	10	11	8	9	14	13
Percentage of yellow-berry			45	50	70	65	95	60	25	75
Percentage of seed shriveling			30	20	25	15	0	0	40	15
Bushels per acre			24.1	33.3	23.2	19.5	22.2	23.0	29.3	25.1
Percentage of stem rust		1,000	15	11	12	8	15	12	15	18
Percentage of yellow-berry			30	60	90	90	35	60	40	40
Percentage of seed shriveling			15	15	15	0	0	20	20	15
Bushels per acre			41.7	20.1	24.4	14.9	15.2	14.0	32.0	31.4
Percentage of stem rust		500	17	13	10	15	12	12	14	15
Percentage of yellow-berry			15	75	98	95	90	60	75	75
Percentage of seed shriveling			40	20	0	0	0	0	15	20
Bushels per acre			23.8	28.3	27.3	14.5	15.1	14.8	34.8	28.0
Percentage of stem rust		None.	16	17	16	11	13	9	15	16
Percentage of yellow-berry			0	70	90	95	95	75	55	75
Percentage of seed shriveling			0	30	0	0	0	0	0	15
Bushels per acre			13.4	22.4	23.1	13.6	12.2	18.3	27.5	33.8
							500	250	500	250
							Potassium chlorid.			
			Fertilizer, kind and amount (pounds) per acre.							

The percentages of stem rust in these plats at Anoka varied but little (Table XV). The percentages of yellow-berry and seed shriveling were affected by fertilizers about as they were in the preceding experiments. The yields were increased by nitrogen much as they were on the Quinn farm. The average yield for all plats receiving applications of sodium nitrate was 27.4 bushels per acre, and for all plats receiving none, it was 16.4 bushels, a difference of 11 bushels in favor of the plats receiving nitrogen. The data obtained from this experiment tend to show that stem rust was affected but little, even though the morphology and physiology of the wheat plants were affected considerably by fertilizers.

GENERAL OBSERVATIONS, 1918 TO 1922

After 1917, observations were made on permanent tenth-acre triplicated plats of the division of soils of the University of Minnesota, at the Crookston substation, and observations were made one year on similar

plats at the Morris substation. The description of the soil at Crookston is given in the discussion of methods. Each plat was sown to Marquis wheat and no attempt was made to produce artificial epidemics of stem rust. Notes on stem rust were taken just before the plants ripened.

The results for 1918 and 1920 are given in Table XVI. Detailed figures are not available for 1919 and 1921. However, there was little observable effect of fertilizers on the development of stem rust. At Morris, in 1921, the wheat in the phosphate plats grew more vigorously and produced a denser stand than that on the other plats. But this seemed to have but little effect on the amount of stem rust, due partly to early maturity. The stand on manured plats was heavy, but the plants ripened so late that there was a slight increase of stem rust.

The differences between rust percentages in various plats in 1918 are not great (Table XVI). When the averages for the three replications are made, the differences are not significant. There is a complete lack of correlation between the percentage of stem rust on the plants and the fertilizers applied to the soil.

TABLE XVI.—*The percentages of stem rust on Marquis in 1918 and 1920 in fertilizer plats at Crookston, Minn.*

Plat No.	Fertilizer per acre.	Percentage of stem rust.	
		1918.	1920.
1	Control	40	50
2	1 ton rock phosphate	30	50
3	8 tons manure + 1 ton rock phosphate	35	50
4	8 tons manure	50	40
5	8 tons manure + 480 pounds acid phosphate	35	50
6	480 pounds acid phosphate	35	50
7	Control	47	70
8	1 ton rock phosphate	50	60
9	8 tons manure + 1 ton rock phosphate	50	15
10	8 tons manure	45	25
11	8 tons manure + 480 pounds acid phosphate	40	50
12	480 pounds acid phosphate	45	30
13	Control	30	50
14	1 ton rock phosphate	45	30
15	8 tons manure + 1 ton rock phosphate	40	20
16	8 tons manure	45	40
17	8 tons manure + 480 pounds acid phosphate	50	50
18	480 pounds acid phosphate	40	25

In 1921, there was a wide variation in percentage of stem rust in certain individual plats, but in the three replications these differences were not consistent, a fact attributed to the very spotted and nonuniform occurrence of the rust, so characteristic of the epidemic throughout the State. Early in July drought checked the spread of rust, and a uniform epidemic

prior to the maturity of the plants could not be expected. The average percentages of stem rust for the three replications are fairly uniform and show no consistent correlations with the fertilizers. A few more plat replications undoubtedly would have made the results just as conclusive as those for 1918.

In 1922, observations were made on a field of winter wheat at the Crookston substation in which several strips across the field had been fertilized with acid phosphate. No difference in growth or maturity of the plants could be observed and there was a perfectly uniform stem rust infection of 10 per cent on all the plats. Observations were made also on plats of Mindum, C. I. No. 5296, a durum wheat, in a four-year rotation series with different rates of manuring. The results are given in Table XVII. The amount of lodging was directly proportional to the amount of manure applied, but the degree of stem rust infection was uniform in all of the plats, at least as late as about two weeks before harvest.

TABLE XVII.—*The effect of barnyard manure on degree of lodging and percentages of stem rust on Mindum in 1922 in the fertilizer plats at Crookston, Minn.*

Amount of manure.	Percentage of plants lodged.	Percentage of stem rust.
None	0	10
4 tons	Trace.	10
8 tons	5	10
16 tons	25	10
16 tons (in 2 applications)	35	10
32 tons	60	10

SIGNIFICANCE OF RESULTS

The average percentage of rust in all plats receiving nitrogen compared with the average percentage in those receiving none gives a fair indication of the resultant differences from the two treatments. The averages, however, may or may not be a true expression of the differences between the two series of plats. If the individual percentages entering into the average are relatively small, it becomes a fairly accurate expression, but, as these increase, the average becomes less and less a reliable expression of the true condition. In considering such a variable as the effect of fertilizers, comparison of two series in an experiment comprising a small number of plats is most availably presented by the method given by Student in Biometrika (*34*). By this method the resulting differences between the treatments are expressed in odds.

In the following discussion of results, the odds have been calculated mainly for the rust percentages and yield, these being the two chief characters under consideration in this paper. The odds are calculated from the results on two adjacent series of plats. The series of four plats receiving 250 pounds of sodium nitrate were adjacent to a series of four receiving none. These two series of homologous plats are used in all of the experiments with commercial fertilizers. They are shown in Table I as plats 3, 10, 17, and 24 for the nitrogen series; and plats 4, 11, 18, and 25 for the adjacent non-nitrogen series. In the case of manured plats in the years 1918 and 1920 (Table XVII) the odds are calculated by comparing

the results from the manured plats with those from the plats which received no manure.

From a general inspection of the data obtained in 1915 (Table III), one can readily determine that the difference in rust percentages on the two series of plats are not great enough to be considered significant. This fact is further demonstrated by the calculated odds of only 4.1:1 for Haynes Bluestem in favor of the nitrogen plats. Mathematicians have shown that in order to be significantly different the odds in favor of one treatment over another ought to be 30:1, which is approximately 3.2 times the probable error. With Iumillo, the odds for rust percentages are 7.4:1 in favor of the plats not receiving nitrogen. The differences obtained on these plats in this experiment, therefore, are not significant, and the only possible conclusion that can be drawn is that the differences in rust percentage caused by the two treatments are negligible. In the case of yield of grain of Haynes Bluestem, the odds are 42.5:1 in favor of the series not receiving nitrogen, indicating a significant difference for this character. The average acre yield for all plats receiving nitrogen is 12.6 bushels, and for all others it is 23 bushels.

In 1916 (Table VI) the calculated odds for stem rust on Haynes Bluestem are 9.2 : 1 in favor of the series not receiving applications of nitrogen, and therefore are not significant. With the hybrid, the odds are 55.5 : 1 in favor of the plats not receiving applications of nitrogen. These odds indicate a significant difference. The average rust percentage on the hybrid, however, for all plats receiving nitrogen and for those not receiving nitrogen are 47.9 and 43.3 per cent, respectively. The calculation of the odds for these two series appears to give a significant difference, but when considered with the average rust percentages for the experiment as a whole, the results are not so convincing. In the case of yield of grain of Haynes Bluestem, the odds are 28.9 : 1 in favor of the series not receiving nitrogen, indicating a fairly significant difference for this character. The average acre yield for all plats receiving nitrogen is 0.77 of a bushel and for all others it is 2.9 bushels.

In the experiment at University Farm in 1917 (Table IX) the calculated odds for rust on Marquis are 7.5 : 1 in favor of the series receiving applications of nitrogen. For the hybrid, the odds are only 2 : 1 in favor of the series not receiving applications of nitrogen. It is evident that the differences in rust percentages on either variety in the two series are not large enough to be significant. In the case of yield of grain of Marquis, the odds are only 11.6 : 1 in favor of the series not receiving nitrogen, and therefore are not significant. The average acre yields for all nitrogen plats is 20.7 bushels and for all others it is 29.9 bushels.

On the plats at the Quinn farm in 1917 (Table XI), the calculated odds for rust on Marquis are 6.8 : 1 in favor of the series receiving nitrogen. For the hybrid, the odds are 8.2 : 1 in favor of the series receiving nitrogen. These odds are too small to be considered significant for either variety. In the case of yield of grain of Marquis the odds are 67 : 1 in favor of the series receiving nitrogen, a very significant difference. The average acre yield for all nitrogen plats is 33.5 bushels and for all other plats is 25.8 bushels. This experiment was made on a rather light soil and the yields were increased by applications of nitrogen, contrasting strongly with the results of the preceding experiments which were on a rather heavy soil on which nitrogen decreased yields.

The calculated odds for height of plants of Marquis are 20.7 : 1 in favor of the series receiving nitrogen. (Table XIII.) These odds are hardly great enough to be considered significant. The differences between the individual plats, however, are quite consistent, and the average height of plants on the plats receiving nitrogen is 39.8 inches and for all others it is 36.2 inches.

The calculated odds for percentage of crude protein (Table XIV) in the seed of Marquis is 21.5 : 1 in favor of the series receiving nitrogen. These are only fairly significant; but here, too, as for height of plants, the differences between the individual plats are small but quite consistent. The average percentage of crude protein for all plats receiving nitrogen is 15.8 compared with 12.9 per cent for all other plats.

On the plats at the Anoka farm in 1917 (Table XV) the calculated odds for rust on Marquis are only 1.6 : 1 in favor of the series receiving nitrogen. For yield of grain, however, the odds are 45.3 : 1 in favor of the series receiving nitrogen. This experiment was made on a light, sandy soil, and the application of sodium nitrate was very beneficial. The average yield per acre for all plats receiving nitrogen is 27.4 bushels and that of all other plats is 16.4 bushels.

Unfortunately, the experiments in 1915 and 1916 with barnyard manure were not laid out so that it would be feasible to arrange the plats in series and calculate odds for significance of difference in results. In 1915 the average percentages of rust for Bluestem and the hybrid on the manured plats was 83 and 38.4 per cent, respectively, compared to 70 and 33 per cent, respectively, for the controls. These rust percentages, which were taken at the time of maturity of the last plat to ripen, appear to indicate significant differences. There is a direct relation to the time of maturity, however, as is illustrated in Tables IX and XVII where, on a given day, say the date of maturity of the first control, there are no differences in the rust percentages in the various plats, but where some of the plats remain green and exposed to infection for a longer period of time, their total percentage of rust infection is bound to increase. In 1915 the average number of days from seeding to maturity was 114.6 compared to the controls with an average of 102.7.

GENERAL CONCLUSIONS

In discussing the effect of fertilizers on the susceptibility of wheats to *Puccinia graminis*, it is necessary to consider what is meant by this susceptibility. Plants may be physiologically resistant, or morphologically resistant, or they may merely be disease-escaping. That there is a true physiologic or protoplasmic basis for resistance to *P. graminis*, probably none will deny. It is well known that this is a genetic character comparable with other hereditary characters. The results of field experiments and observations suggest also that plants sometimes may be resistant on account of morphologic characters. Certainly, plants with a large amount of woody tissue are not likely to be so severely rusted as are those with a larger proportion of succulent tissue because the rust fungus requires the latter for growth. But this morphologic resistance is quite different from true protoplasmic resistance; it is only a mechanical limitation on the spread of the rust mycelium. Under normal conditions, in some varieties, it undoubtedly is due to inherited genetic factors, while in other varieties it may be due to environmental conditions. It also is well known that early maturing varieties may ripen

early enough to escape infection to some extent. The differences between these kinds of resistance, on the one hand, and those phenomena on the other hand which clearly can not be defined as resistance, should be kept distinctly in mind, as they probably have been confused frequently in field studies similar to those reported in this paper. This confusion, together with the fact that opportunities for infection often are affected considerably by density of stand and other factors, has given rise to the view that resistance to rust can be modified easily by changing environmental conditions.

Resistance of wheat varieties to *Puccinia graminis* may not be rigidly immutable. One would not expect it to be. As plant characters are the resultant of the interaction of genetic and environmental factors, fluctuations are bound to occur. Otherwise every plant of the same pure line should always be exactly like all the other plants of that pure line. But such is not the case. Neither are all of the individuals of a pure-line wheat variety affected equally severely by stem rust, even when they are growing under apparently identical environmental conditions. Yet, two plants seldom if ever grow under identical conditions. It is necessary, therefore, to distinguish as clearly as possible between the effects of genetic factors and those of environmental factors. The results presented in this paper lend practically no support to the opinion that the genetic resistance of wheat varieties is changed by fertilizers. The type of infection remained the same on susceptible varieties [11] like Glyndon Fife, Haynes Bluestem, and Marquis and on resistant varieties like Iumillo and the resistant hybrids, regardless of fertilization. More and larger uredinia sometimes developed on wheat in some fertilizer plats than on that in others. This seemed to be due, however, to increased opportunity for infection or to structural changes in the plants which made it possible for the rust mycelium to spread more extensively.

Morphologic resistance may be changed somewhat by fertilizers. It is clear from the data obtained that the height of the plants and stiffness of straw are affected greatly by different fertilizers. The stiffness of straw, of course, is an index of the amount of supportive tissue in the plant. *P. graminis* and other cereal-rust fungi grow almost entirely in chlorenchymatous tissues. If the fertilization is such as to increase the amount of mechanical tissue, the rust mycelium can not thrive as well as it can in plants containing large amounts of chlorenchyma. But the writers found little evidence that, under field conditions, anything approaching normal fertilization changed the morphologic resistance to stem rust greatly. There was much more convincing evidence that fertilizers affected the final degree of rustiness of plants by hastening or delaying ripening and by affecting the stand.

The degree of infection by orange leaf rust seemed to be increased by heavy applications of nitrogen in some plats, but the rust was not abundant enough in most of them to make it possible to draw final conclusions.

Any fertilizers which prolong the growing period and thus delay maturity are likely to increase indirectly the amount of rust. Late maturing plants are exposed to inoculation longer than are those which mature early. A few days' difference between the dates of ripening of two fields of wheat may have a marked influence on the amount of rust which

[11] This statement applies to susceptibility to the biologic forms used and to the general reaction o the varieties in the field at University Farm, St. Paul.

may develop. In the hard red spring wheat region the stem rust epidemic is likely to come just before harvest. Early ripening fields often escape great damage, whereas those which mature a week later may be damaged severely. Under fairly normal growth conditions for wheat, barnyard manure and other nitrogenous fertilizers may delay ripening for a few days or even for two weeks or more, depending on the soil type and amount of fertilizer used. It has been shown that the percentage of stem rust often could be correlated directly with the time required for maturity. The conclusion is that there is more rust on the late-ripening wheat than on that which ripens earlier. For practical purposes, therefore, nitrogen has been conducive to rust, but it has not increased susceptibility in the strict sense; it has only prolonged the growth period so as to expose the plants longer to the rust danger. But the effect may be just the opposite. In 1916, for instance, when the weather was very hot and dry after the wheat headed, the plants in the nitrogen plats actually ripened earlier than those in the other plats. It is true that it was not normal ripening, the plants being practically "burned up," but the natural result was that the rust was checked also.

Fertilizers also may make it possible for the rust fungus to infect plants more easily. When nitrogenous fertilizers increase the density of stand, succulence of the plants and consequent lodging, moisture is retained longer in the field than in those in which the stand is not so dense and the plants remain upright. The minimum incubation period for the uredinial stage usually is about six hours. There is an increase in the number of germ tubes which enter the plants as the incubation period is increased. Assuming that two fields have been inoculated equally heavily, other things being equal, the heavier infection will occur in the field in which moisture is retained longer.

It is perfectly clear from the data obtained that the percentage of stem rust on wheat plants does not necessarily indicate the amount of damage which will be done. When wheat is grown in properly fertilized soil it often yields well in spite of heavy attacks of rust, while that growing in soil with unbalanced fertilization may yield poorly regardless of the degree of severity of rust. It seems very likely, therefore, that much of the damage often attributed to rust when wheat is growing in soil containing an overabundance of nitrogen, actually is due to the direct effect of the fertilizer on the plants. It is quite likely also that a given amount of rust may injure plants too heavily fertilized with nitrogen more than it does plants growing in soil containing well balanced soil nutrients. This may be due partly to increased water requirement, still further increased by rust infection, and partly to weak straw which may be weakened still more by rust. The rational thing to do is to supply the soil with the nutrients which the plants need. Nitrogen decreased yields consistently on University Farm soils, but on the lighter soils on the Quinn farm and at Anoka it increased them.

The final conclusion seems to be justified that wheat can not be predisposed easily to *Puccinia graminis*, if we mean by predisposition the action of environmental factors in rendering a variety more susceptible than it normally would be. Nitrogen apparently may predispose plants by increasing succulence, density of stand and lodging, by delaying maturity and by decreasing yields on account of these factors, and sometimes by premature ripening in hot, dry weather. But the actual predisposing action apparently is of relatively minor importance. Since the rust fungi are highly specialized obligate parasites, a mere weakening of the

host not only does not usually increase susceptibility, but may decrease it. The relationship between host and parasite is so intimate that it is difficult to generalize about it. As a rule, however, plants which grow normally are likely to yield best, regardless of rust. And the yield must be the real test of the value of fertilizers.

For practical purposes much can be done to increase yields when stem rust is prevalent. The old principle of avoiding excessive applications of nitrogenous fertilizers is sound. But some soils need them and on such soils wheat will yield better when they are supplied. Acid phosphate and potassium often are valuable in increasing stiffness of straw and promoting early maturity. It is useless to add them, however, to soils which do not need them, or in which they do not become available. The opinion that they have an actual immunizing effect against stem rust does not seem to be justified, although the indirect effect may be very great. The problem of fertilization, in order to reduce losses from rust, varies with soil type and climate. The writers are forced to the conclusion that, while the direct effect of fertilizers on the susceptibility of wheat to stem rust under field conditions is slight, the indirect effect on rust and the direct effect on yield may be very great. Give the soil those fertilizers which the wheat needs, but avoid too much nitrogen, add potash and phosphates judiciously, and the best results are likely to be obtained.

SUMMARY

Experiments and observations, covering a period of eight years, have been made to determine the effect of artificial and natural fertilizers on the amount of stem rust developed on susceptible and resistant varieties of wheat, when grown on several soil types in different parts of Minnesota. The plants were grown under heavy artificially induced epidemics, and under natural field epidemics.

The effect of barnyard manure, clover soil, alfalfa soil, and cabbage soil, with and without fertilizers, was tried in the field. In addition, various amounts of sodium nitrate, acid phosphate, and potassium chlorid or potassium sulphate, alone and in combinations with each other, were added to soils to determine the effect on infection of wheat by rust.

The degree of physiologic susceptibility of susceptible and resistant varieties apparently was not changed directly by the use of different fertilizers, although morphologic resistance may be changed slightly.

Plants growing in plats heavily fertilized with nitrogenous manures sometimes were more heavily attacked by stem rust than those in other plats. This apparently was an indirect effect due to increased density of stand and delayed maturity, which made conditions for infection more favorable and lengthened the time during which the plants could become infected. In hot, dry weather, plants heavily fertilized with nitrogen may burn out, thus indirectly causing a decrease in the amount of rust.

There is some evidence that the amount of orange leaf rust may be increased by the use of nitrogenous fertilizers.

The direct effect of fertilizers on the character of plant growth and yields seems to be much more important than their effect on the severity of stem rust.

The date of maturity, degree of lodging, crinkling, shriveling of seed, percentage of yellow-berry, and yield of straw and grain may be affected profoundly by different fertilizers.

On some soils, and under certain weather conditions, heavy fertilization with nitrogen decreases yield greatly by increasing the proportion of straw to seed, by inducing lodging and burning out. This probably has been confused with rust injury.

On soils deficient in nitrogen, barnyard manure and nitrates increased yields without increasing the severity of attack by stem rust.

Neither acid phosphate nor potassium counteracted the harmful effects of excessive fertilization with nitrogen on some soils.

On properly fertilized soil wheat yielded well in spite of heavy attacks of stem rust.

While the direct effect of fertilizers on the development of stem rust seems to be slight, there is sometimes a profound indirect effect. It is advisable, therefore, to avoid excessive fertilization with nitrogen and to use phosphates and potassium fertilizers on those soils which need them. Rust damage can be reduced in this way.

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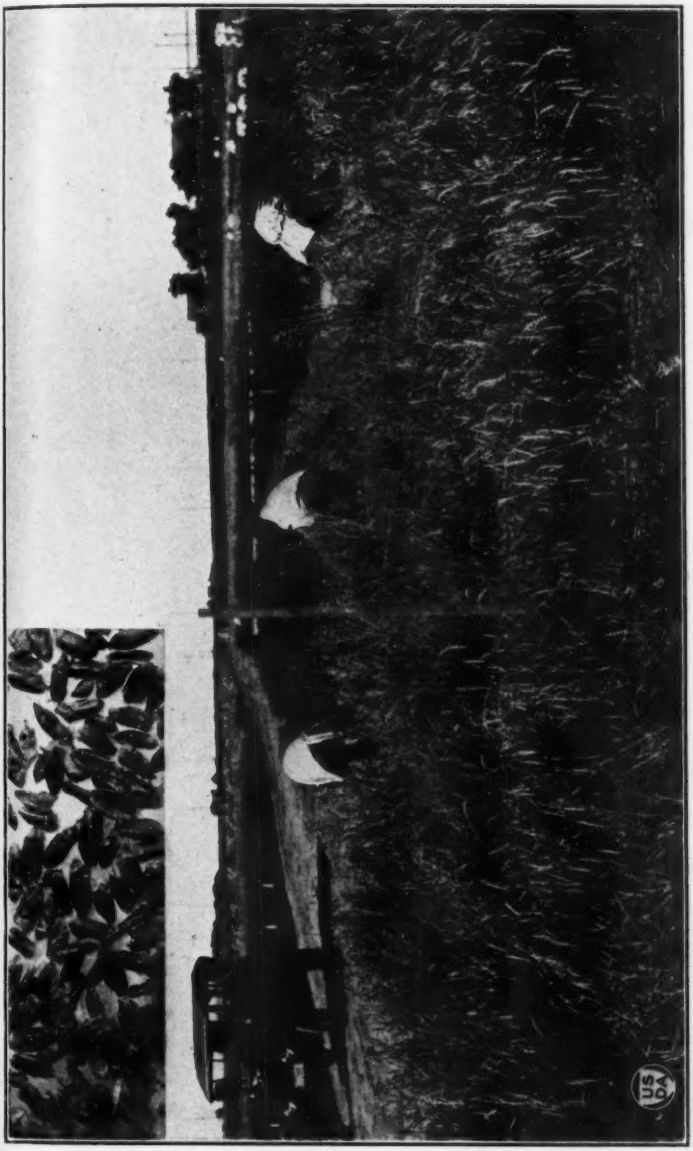
PLATE 1

Photograph of plat 8 in the commercial fertilizer series in 1917 on University Farm (see Table IX). Marquis on the left and hybrid on the right. This plat had received acid phosphate at the rate of 1,000 pounds per acre in 1916 but none in 1917. It had received sodium nitrate at the rate of 1,000 pounds per acre in 1917. Note the very severe lodging. This is characteristic of the effect of nitrogen on University Farm. Note also the badly shriveled seed from the Marquis. This variety showed 60 per cent of stem rust, and the yield was 15.8 bushels per acre. Compare with Plates 2 and 3.



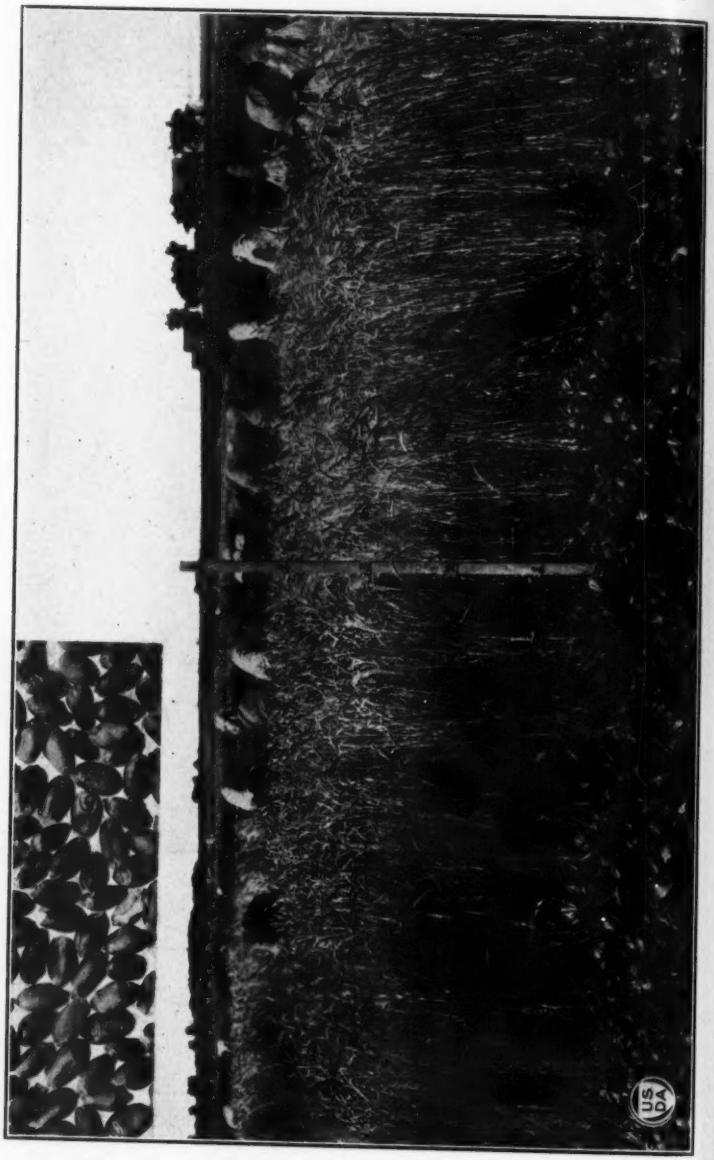
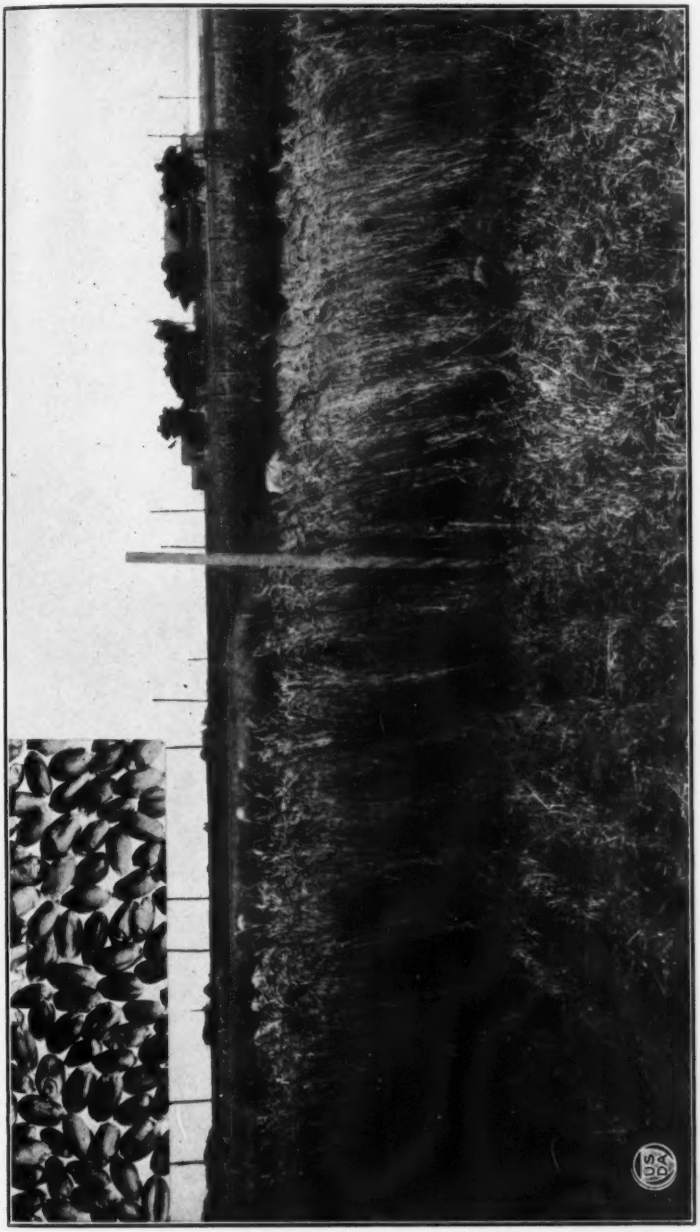


PLATE 2

Photograph of plat 21 of the commercial fertilizer series in 1917 on University Farm (see Table IX). Marquis on the left and hybrid on the right. This plat had received potassium chlorid at the rate of 250 pounds per acre and acid phosphate at the rate of 500 pounds per acre in 1916, but no fertilizer in 1917. Note the uniform stand and freedom from lodging, in sharp contrast with the condition shown in Plate 1. Note also the plump kernels of Marquis compared with the very shriveled kernels shown in Plate 1. The percentage of stem rust was 27 per cent on Marquis and 2 per cent on the hybrid, and the yield of Marquis was 32.6 bushels per acre. The wheat in many of the plats fertilized with acid phosphate and potassium chlorid was like this. The kernels were plump and the yield was satisfactory in spite of the fact that on some of them the rust infection in previous years was from 50 to 85 per cent.

PLATE 3

Photograph of plat 26 of the commercial fertilizer series in 1917 on University Farm (see Table IX). Marquis wheat on the left and hybrid on the right. This plat received potassium chlorid at the rate of 1,000 pounds per acre in 1916 but no fertilizer in 1917. The rust infection was 32 per cent on Marquis and 7 per cent on the hybrid. The yield on Marquis was 33.2 bushels per acre. Note the plump kernels of Marquis and compare with those shown in Plate 1.



MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE RESISTANCE OF WHEAT TO PUCCINIA GRAMINIS TRITICI ERIKSS. AND HENN.[1]

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INTRODUCTION

Many conflicting statements have been made regarding the nature of resistance of wheat varieties to *Puccinia graminis tritici* Erikss. and Henn. It is clear, also, that there are many different opinions regarding the degree to which this resistance can be changed. For this reason it seemed desirable to investigate the factors which affect the virulence of rust attacks.

There are two conceivable reasons for the resistance of plants to fungous attack—morphological peculiarities and physiological resistance.

There may be mechanical obstacles to the entrance of the germ tubes of the rust fungus into the tissues of the host. While the host cells may be quite susceptible after the germ tubes once have entered, it is quite conceivable that so few may enter that the plant is protected against heavy attack. The number of hairs on the surface of the plant, and the number, size, disposition, and peculiarities of movement of the stomata, conceivably could exert a considerable effect upon the entrance of the germ tubes.

If the pathogene is unable to obtain proper food materials from a certain plant, even after it has entered, the possibility of its parasitizing that plant is precluded, of course. Furthermore, if toxic substances occur in the tissues of the plant, the pathogene will be weakened or killed. It is possible, however, that the cells of certain tissues may be quite susceptible to the attacks of a pathogene but that the number of these cells may be so limited as to make it impossible for the organism to develop extensively. A plant therefore may be physiologically susceptible and morphologically resistant. There appeared to be good evidence from field observations that certain varieties of wheat apparently were resistant to certain biologic forms of *P. graminis* merely because there were not sufficient tissues in the host plant in which the rust parasite could develop. If the plant develops a large amount of woody tissues, it is clear that the area in which rust hyphae can grow is rather limited.

It is well known that the morphology of wheat plants may be affected by temperature, light, moisture, and soil nutrients. Just to what extent environmental factors could affect the development of rust indirectly by affecting the structure of the plant has not been shown. It frequently

[1] Accepted for publication Nov. 19, 1923. Cooperative investigations between the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agriculture of the University of Minnesota. Published with the approval of the Director as Paper No. 401 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer takes pleasure in acknowledging his indebtedness to Dr. E. C. Stakman, Professor of Plant Pathology, University of Minnesota, and Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, for suggesting the problem and for much helpful criticism during its prosecution.

has been observed that varieties which may be susceptible in the seedling stage apparently are resistant in the field. That is, the rust does not develop on them sufficiently to do any damage. The indications are that either the germ tubes fail to enter the host, or, if they do, the mycelium for some reason can not develop extensively. Attention has been focused on this phase of the problem particularly since the discovery of numerous biologic forms of *P. graminis tritici.*

The specific problems which the writer undertook to investigate, therefore, were the following: (1) Are the morphological differences in different varieties of wheat sufficiently great to affect materially the entrance of germ tubes? (2) After the germ tubes have entered, are there structural and physiological peculiarities which account for the inability of certain biologic forms to develop in certain varieties of wheat? (3) Can these structural and physiological characters be altered sufficiently by environmental factors to change the reaction of the host to the rust parasite?

HISTORICAL REVIEW

Much of the literature on the subject of rust resistance has been reviewed elsewhere, so only a brief review will be given of those papers which bear definitely on the problems at hand.

The relation of the morphology of the host to rust resistance has been studied by several investigators. Sappin-Trouffy (*47*) [3] stated that the mycelium of *Puccinia graminis* is entirely localized in the chlorophyll-bearing parenchyma, so that it can grow readily only longitudinally in the stem, being prevented from extensive radial spread by the hemispherical sheath of sclerenchyma fibers. The natural result is the formation of linear pustules.

Cobb (*8*) thought that the degree of rust resistance of wheat varieties was correlated with the tensile strength of the leaves, the ratio of sclerenchyma to chlorenchyma, the amount of waxy bloom, the number and size of stomata, and the number and length of leaf hairs. Petermann (*40, p. 15–16*) suggested that silica increased the strength of cell walls and their resistance to puncture, thus increasing resistance. Farrer (*13*) was of the opinion that wheat plants with narrow erect leaves and thick epidermis were likely to be resistant. Ward (*57*), Eriksson and Henning (*12*), and Biffen (*6*), on the other hand, concluded that external morphology had little effect on resistance to various rust fungi.

Ward (*57*) found that *Puccinia glumarum* entered susceptible and resistant hosts equally well, but that the mycelium could not develop normally in the latter. The hyphae soon died in the resistant host, either on account of starvation or poisoning. Gibson (*16*) showed that germ tubes of rust fungi easily gained entrance through the stomata of many plants, but that the hyphae could produce haustoria and develop subsequently only in susceptible forms. Marryat (*34*) in general confirmed the observations of Ward and Gibson and showed further that the cells of wheat varieties resistant to *P. glumarum* were killed by the rust hyphae, which then also died. Stakman (*51*), Allen (*2*), and Newton (*37*) found that essentially the same thing was true of *P. graminis.* Allen (*1*) further showed that the size and shape of the guard cells may be a factor in the entrance of the germ tube.

[3] Reference is made by number (italic) to "Literature cited," p. 408.

Ward (*58*), Arthur (*4*), and Stakman (*50*) emphasized the importance of the effect of the physiologic condition of the host plant on the development of rust. Their general conclusion was that the vigor of the parasite is directly proportional to the vigor of the host. Biffen (*6*) concluded that the virulence of *P. glumarum* is dependent on the amount of fertilizer used.

It has long been considered that the plane of metabolism of the host profoundly influenced the degree of development of rust. See Raines (*42*). See also paper of Stakman and Aamodt in the present number of this Journal. The opinion has been prevalent that heavy applications of nitrogen increased the susceptibility of cereals to rusts. See Sorauer (*48*), Hiltner (*23*), Comes (*10*), Spinks (*49*), Little (*29*), Bolley (*7*), Anderson (*3*), Remer (*43*), Freeman (*14*), Cobb (*8*), Stakman and Aamodt (*53*). It has also been thought that potassium and phosphates rendered them more resistant. See Montemartini (*35*), Müller and Molz (*36*), Sorauer (*48*), Hiltner (*23*), Comes (*10*), Spinks (*49*), Remer (*43*). On the other hand, Pantanelli (*39*) found that excess phosphate in relation to nitrogen increased resistance only when it checked the growth of the host plants. Stakman (*52*) states that large amounts of nitrogenous fertilizers, particularly on soils which do not need them badly, will permit greater rust damage. Plants so fertilized have a weak straw which crinkles badly when rust attacks it. The development of a stiff straw is desirable, and plants fertilized with potassium or phosphate fertilizers usually yield better in bad rust years than those heavily fertilized with nitrogen.

As to the influence of certain physicochemical properties of the sap of the host plant upon rust development, Comes (*10*) concludes that resistance of wheat to *P. glumarum* is increased by superphosphate fertilizer but is weakened by nitrogenous fertilizer, and suggests that this increased resistance might be due to an increase in the acidity of the sap. He states that the sap of "Rieti" wheat is more acid than that of any other Italian wheat. This variety is particularly resistant to rust. Kirchner (*27*) and Henning (*22*) also call attention to the importance of high acidity of the cell sap in resistance to rust. Gortner (*17*) reports that excess fertilization with sodium nitrate decreased the hydrogen-ion concentration in Haynes Bluestem wheat. Grimaldi (*19*) and Hurd (*24*) question the importance of the acidity of the cell sap in resistance of wheat to rust, pointing out that there is no positive correlation between the two. The latter shows that environmental conditions may cause greater differences in the acidity of the cell sap of a single variety than usually occur between different varieties grown under the same conditions. Pantanelli (*39*) states that the organs of the wheat plant most susceptible to rust are those richest in sugars, acids, and in soluble compounds of phosphorus and nitrogen.

Mains (*32*) and others have shown that the carbon metabolism of the host is important in the development of rust. Kirchner (*27*) and Henning (*22*) give tables of the sugar content of the saps of resistant and susceptible varieties. These tables indicate that the concentration of glycose or reducing sugars is higher in the more susceptible varieties.

EXPERIMENTAL DATA

Studies have been made on the entrance of germ tubes of *Puccinia graminis* into the host, the morphology of the wheat plant as related to rust resistance, the relation of nutrient salts to the development of stem

rust, and the relation of the physicochemical properties of the plant sap to rust resistance.

ENTRANCE OF THE GERM TUBES OF PUCCINIA GRAMINIS

The factors influencing the entrance of the germ tubes of *Puccinia graminis* into the host, which have been studied by the writer, are the number of leaf hairs, the number of stomata, the size and movement of stomata, and the relation of the formation of appressoria to subsequent infection.

Relative Number of Leaf Hairs

In order to compare the number of hairs on different varieties of wheat, the leaves of seedlings, 12 days old, were placed in aceto-alcohol (glacial acetic acid and 95 per cent alcohol, 1 part each) for several days. The pigments were so thoroughly removed by this process that the leaves could be mounted entire on a slide and observed with direct light under the binocular microscope. The hairs on leaves thus treated can be seen distinctly and can be counted easily.

As shown in Table I, there is a consistent difference in the number of hairs on the leaves of the five varieties of wheat used in this study. Kota always has the most and Little Club the fewest. Observations on leaves from the field at heading time show the same general differences. No hairs were found on the culms of any of the varieties observed.

TABLE I.—*Comparative number of hairs on the two surfaces of leaves of five wheat varieties*

[Average of 10 microscopic fields (2.138 sq. mm.).]

Variety.	On midrib.		Edge of blade.		Average for leaf.	Average both surfaces.
	Distance from tip.		Distance from tip.			
	1 inch.	2 inches.	1 inch.	2 inches.		
Kota, C. I. 5878:						
Lower surface	106	98	108	99	103	102.5
Upper surface	105	101	107	94	102	
Khapli, C. I. 4013:						
Lower surface	61	56	85	80	70	57.5
Upper surface	54	47	53	46	45	
Kanred, C. I. 5146:						
Lower surface	20	29	24	32	26	28.0
Upper surface	35	27	31	28	30	
Marquis, C. I. 3641:						
Lower surface	5	4	8	5	6	13.5
Upper surface	23	16	24	22	21	
Little Club, C. I. 4066:						
Lower surface	1	6	1	8	4	9.0
Upper surface	12	14	11	20	14	

The five wheat varieties listed in Table I were inoculated on the first leaf with urediniospores of *Puccinia graminis tritici*, Form XXXIII, and allowed to incubate 48 hours, when they were placed on the greenhouse bench. Two days later some of the inoculated leaves were removed and cleared in aceto-alcohol for several days. The entire leaf was then immersed in Pianeze stain for one minute and then washed thoroughly in dis-

tilled water. The leaf was then mounted entire on the slide and examined with direct light under the microscope. It was observed that the germ tubes often came in contact with hairs and entwined about them. It is believable that this might reduce the number of germ tubes which are able to reach the stomata and form appressoria. Table V shows that on Kota, the variety with the largest number of leaf hairs, the actual number of appressoria observed on the average leaf is lower than that on varieties with fewer hairs, although enough appressoria were developed to permit infection. In these experiments, therefore, the leaf hairs could not be considered as a serious obstacle to entrance. If the inoculum is abundant, and other factors are favorable, the number of hairs on wheat leaves probably has very little effect on the final degree of infection. On the other hand, if the opportunities for germination and germ-tube development are not particularly favorable, it is possible that a large number of hairs may reduce the number of infections somewhat.

Relative Number of Stomata

As the germ tubes of *Puccinia graminis* must enter the wheat plant through the stomata, it seems possible that entrance would be easiest in those varieties in which there are many stomata per unit area of surface. Thus it would be important to know the comparative number of stomata on varieties which show marked differences in resistance. The varieties under investigation were grown in adjacent rows in the plant pathology field plots at University Farm, St. Paul, Minn.

The relative number of stomata on the neck of the stem was first determined. Three varieties were selected: One resistant in the field, Kota, C. I. 5878; one susceptible, Little Club, C. I. 4066; the third moderately susceptible, Marquis, C. I. 3641. These host reactions are based on general behavior to stem rust at University Farm under conditions of an induced epiphytotic. At the time the grain was in the hard-dough stage the peduncles, from the upper node to the rachis, were cut and killed in aceto-alcohol.

Table II shows the actual counts made on strips of epidermis from several stems of each variety, and represents the average number in 10 microscopic fields. The number of stomata on Little Club, the susceptible variety, was considerably higher per unit area than that on the other varieties.

Table II.—*Number of stomata per microscopic field (1.150 sq. mm.) on peduncles of three wheat varieties*

Variety.	Distance from rachis.		Average.
	0.5 inches.	2.5 inches.	
Little Club, C. I. 4066	156	112	134
Marquis, C. I. 3641	112	69	90
Kota, C. I. 5878	95	41	68

In Little Club, also, the bundles of sclerenchyma fibers are far apart; in Kota they are closer together and often allow for only a single row of stomata. (Pl. 1, C.)

To determine the relative number of stomata on the leaf, seedlings of wheat varieties were grown in the greenhouse. When the seedlings were 10 days old, the first leaf of several plants of each variety was cut off near the base and placed in aceto-alcohol until clear. The entire leaf was then mounted in aceto-alcohol and observed through strong transmitted light. Counts were made both on the midrib and at the outside of the leaf, 1 inch and 2 inches from the tip.

TABLE III.—*Average number of stomata in 10 fields, each containing 2.138 sq. mm., from both surfaces on different parts of the leaves of five wheat varieties*

Variety.	On midrib. Distance from tip.		Edge of blade Distance from tip.		Average for leaf.	Average both surfaces.
	1 inch.	2 inches.	1 inch.	2 inches.		
Khapli, C. I. 4013:						
Lower surface	56	57	52	49	54	66
Upper surface	69	80	78	87	78	
Kanred, C. I. 5146:						
Lower surface	37	47	42	45	43	64
Upper surface	70	78	71	81	75	
Marquis, C. I. 3641:						
Lower surface	52	34	45	28	40	61
Upper surface	79	78	92	84	83	
Little Club, C. I. 4066:						
Lower surface	44	50	44	38	44	61
Upper surface	70	81	73	89	79	
Kota, C. I. 5878:						
Lower surface	36	35	32	33	34	45
Upper surface	56	55	59	56	56	

TABLE IV.—*Size of stomatal apertures on seedling leaves of different wheat varieties in the greenhouse, at different times in a winter day with bright sunlight from 9.45 a. m. to 4.35 p. m., at University Farm, St. Paul, Minn.*

Variety and data on stomatal openings.	Time of day.				
	9.15	11.15	1.15	3.15	5.15
Little Club, C. I. 4066:					
Aperture in micra	32.1 x 0.5	34.3 x 4.35	40.3 x 7.4	38.9 x 7.8	Closed.
Percentage open	1	32	100	100	0
Khapli, C. I. 4013:					
Aperture in micra	22.6 x 0.5	22.6 x 0.5	22.6 x 0.5	24.2 x 0.5	22.6 x 0.5
Percentage open	1	60	75	20	5
Kanred, C. I. 5146:					
Aperture in micra	34.2 x 0.5	35.9 x 5.1	39.4 x 6.3	40.5 x 5.0	Closed.
Percentage open	60	95	95	95	0
Mindum, C. I. 5296:					
Aperture in micra	23.5 x 0.5	28.1 x 1.0	31.3 x 0.9	26.1 x 0.7	31.3 x 0.5
Percentage open	40	80	10	10	5
Marquis, C. I. 3641:					
Aperture in micra	Closed.	33.0 x 1.3	33.0 x 1.3	Closed.	Closed.
Percentage open	0	1	1	0	0
Kota, C. I. 5878:					
Aperture in micra	35.0 x 0.5	36.9 x 2.1	35.1 x 2.9	35.1 x 3.1	Closed.
Percentage open	20	20	70	1	0

There is no great difference in the number of stomata on the different varieties: Kota always has the least, and Khapli consistently the most. (Table III.) The difference in the number of stomata on these varieties seems unrelated to the degree of resistance to stem rust in the greenhouse. Khapli, the most resistant wheat used for determining biologic forms of rust at University Farm, has the largest number of stomata. What appears to be more pertinent to the problem is the frequency and the extent to which the stomata open.

Size and Movement of Stomata

Allen (*1*) suggested that the inability of many germ tubes to gain entrance into Kanred wheat might be due to the size and shape of the stomatal aperture.

The writer compared the size of the aperture of stomata of five wheat varieties. The method employed by Lloyd (*30*) was used. Epidermis strips were placed immediately into absolute alcohol, and then studied under the microscope. Check observations were made by fastening growing leaves to the substage of the microscope and observing the stomata direct. Measurements made in this manner agreed entirely with those from epidermis strips placed in alcohol.

It is obvious from these observations that the stomatal slits of Kanred wheat are open to a much greater extent than would be expected from Allen's preliminary report. These data are shown in Table IV.

Allen (*1*) found that only about 10 per cent of the germ tubes of a form from which Kanred is immune entered this variety, while about 30 per cent of the germ tubes of a form to which Kanred is susceptible entered. The following explanation was offered: "The presence of the appressorium might act as a stimulus by mere contact, by altering the gaseous exchange through the stoma or disturbing the moisture relations, by exerting a possible toxic influence upon the guard cells, or by its presence shutting off some of the light from the guard cells. It is at least conceivable that the guard cells might be sensitive to the appressorium and remain closed, thus excluding the fungus." She also observed that in Baart, a variety very susceptible to most biologic forms of *P. graminis tritici*, only 67 per cent of the germ tubes which formed appressoria entered.

Newton (*37*) has studied the behavior on Kanred of the germ tubes of a biologic form which fails to produce pustules or flecks on this wheat variety, and concludes that approximately 30 per cent of the germ tubes which form appressoria enter. This difference in the observations of different investigators strongly suggests that conditions during the incubation period influence the number of entries which the fungus is able to make. It seems probable, therefore, that the conditions which influence the opening of the stomata, both before and after appressoria are formed, may have considerable effect on entrance.

Loftfield (*31*) points out that the stomata of cereals rarely are all open to their maximum at the same time. Even under the most favorable field conditions all the stomata are open only for one or two hours each day, the tendency in cereals being to operate with many closed stomata. He further observed that on many days the stomata of wheat plants do not open at all.

On the two days prior to the date of observations, careful examination showed that the stomata of the six wheat varieties used in the experiment

presented in Table IV failed to open. These days were very dark, there being no sunlight whatever. It seems that stomatal movement not only varies with different varieties of wheat, but varieties themselves evidently do not respond to a variable in exactly the same way.

Experiments therefore were made to ascertain whether the stomatal behavior in any particular variety of wheat is an important factor in determining the number of stem-rust infections which result from a given number of spores germinating on the leaf surface.

Relation of Formation of Appressoria to Subsequent Infection

Seedlings of five wheat varieties which behave quite differently toward several biologic forms of *P. graminis* were heavily inoculated with viable urediniospores and placed in humidity chambers for 48 hours. The plants then were placed on the greenhouse bench for 48 hours. A number of leaves then were taken at random from each variety and cleared several days in aceto-alcohol. The remaining leaves were left in order to ascertain the number of uredinia which would develop. The cleared leaves then were submerged one minute in Pianeze stain, and washed in water to remove the excess stain. The leaf tissue which had been cleared by the aceto-alcohol did not take the stain, but the germinating spores, germ tubes, and appressoria on the leaf surface were stained purple red. The entire leaf was then mounted, and the germ tubes on its surface were observed in direct light. The results are given in Table V.

Table V.—*Relation between number of appressoria of Puccinia graminis produced on the wheat leaf and subsequent infection*

Wheat variety.	Percentage of urediniospores germinating on leaf.	Average number of appressoria per leaf.	Number inoculated plants infected.[a]	Average number of uredinia per leaf.
Inoculations with biologic form XVIII				
Kanred, C. I. 5146	Abundant; approximately 50 per cent.	17	7/7	3.2
Little Club, C. I. 4066		20	10/10	2.8
Kota, C. I. 5878		15	3/3	2.1
Marquis, C. I. 3641		19	4/4	2.7
Khapli, C. I. 4013		20	3/3	4.6
Inoculations with biologic form XXVII				
Kanred, C. I. 5146	Abundant; approximately 50 per cent.	32	0/7	0
Little Club, C. I. 4066		44	1/1	2.9
Kota, C. I. 5878		17	0/3	0
Marquis, C. I. 3641		27	1/4	2.3
Khapli, C. I. 4013		23	1/3	4.2

[a] The denominator indicates the number of leaves artificially inoculated, and the numerator shows the number which became infected.

The degree of infection produced by biologic Forms XVIII and XXVII on the wheat varieties given in Table V is summarized below. These data are based on Table IV, Technical Bulletin No. 8, Minnesota Agricultural Experiment Station.

Wheat variety.	Degree of infection.	
	Biologic Form XVIII.	Biologic Form XXVII.
Kanred, C. I. 5146	Very heavy	No infection.
Little Club, C. I. 4066	do	Very heavy.
Kota, C. I. 5878	do	No infection.
Marquis, C. I. 3641	do	Weak.
Khapli, C. I. 4013	Very weak	Do.

On some leaves observed in the foregoing study, as many as 60 appressoria were seen, and where the spore germination was profuse there never were fewer than 15. As relatively few uredinia developed, there appeared to be but little correlation between the number of appressoria formed and the number of subsequent infections. For example, it frequently has been observed that even on the completely susceptible Little Club, numerous appressoria may be observed on leaves which fail to become infected. It seems improbable that the germ tubes entered, because antagonism between the host cells and the parasite, which conceivably could account for lack of infection, never has been observed in this variety.

It is probable that the stimuli for the formation of appressoria and for subsequent entrance of the germ tube are entirely different. The formation of appressoria may be due to thigmotropism, because they often are formed in depressions between epidermal cells and in other places where they come in contact with some object. Ward (*59*), Robinson (*44*), Fromme (*15*), and Mains (*32*) noted that the germ tubes of certain rust fungi tended to be negatively heliotropic; while Balls (*5*) suggested that a hydrotropic response might account for entrance. The germ tubes might grow toward the stomatal opening, either on account of a negatively heliotropic or a positively hydrotropic response, or both; but the tubes probably can not enter if the stomata are closed tightly.

According to Allen (*1*), only 67 per cent of the germ tubes which form appressoria enter the very susceptible host, Little Club. If only two-thirds of the germ tubes forming appressoria enter a very susceptible host under favorable conditions, we are not justified in emphasizing the relative number of germ tubes which enter from appressoria formed on resistant hosts. Before the results of different experiments can be compared, it will be necessary to control all the factors which influence the movement of stomata during the incubation period.

It is quite possible that neither the number nor the size of the stomata greatly influences the entrance of the germ tubes. It is probable that the degree and duration of opening are more important. If the stomata are fairly wide open for some time the germ tubes may enter easily, but if they remain closed, there is no evidence that the tubes can force their way into the substomatal chamber. On the other hand, there is abundant evidence to show that even on the most susceptible varieties spores

may produce abundant germ tubes which form appressoria over the stomata and yet do not enter. In these cases it is possible that the stomata may not have opened at all.

It appears probable that entrance in itself, so far as greenhouse studies are concerned, is a factor in the resistance of most wheat varieties only when the stomatal movement is such as to effect complete closure of the aperture. The very fact that many biologic forms regularly enter Kanred seedling leaves under greenhouse conditions is sufficient evidence that the failure of some forms to produce infection can not be due to the small size of the aperture. It seems more probable that the forms which do not enter may have a specific physiological effect upon the guard cells, which respond by complete closure, or that when entrance is effected the germ tubes are destroyed before they can produce any easily visible effect upon the host.

MORPHOLOGY OF THE WHEAT STEM AS RELATED TO RUST RESISTANCE

During recent years the idea has been prevalent that rust resistance was not due to morphology of the host. But it never has actually been demonstrated that the morphology of the wheat stem bears no relation to the development of *Puccinia graminis*. On the other hand, there are certain suggestions in the early literature that the internal structure of the stem may determine in some measure the extent of spread and development of the fungus. This is particularly evident in the plates and figures by Sappin-Trouffy (*47*) and Eriksson and Henning (*12*).

P. graminis causes the greatest injury when it attacks the portion of the stem or peduncle immediately below the rachis, known as the neck. It is evident from field observations that the necks of all varieties are not uniformly attacked by stem rust. Plate 1, F and G, shows two varieties of wheat, one quite susceptible and the other generally resistant to stem rust, in the field plots at University Farm, St. Paul.

The variety shown in Plate 1, F, is Kota, C. I. 5878, which is generally considered resistant in the field. It is attacked by stem rust, however, and normal linear uredinia are produced. These may be as much as a centimeter in length. Usually the uredinia are separate and quite distinct to the eye. Even when the infections are numerous, it is not difficult to make out the characteristically linear individual uredinia. The spores produced are quite normal.

Plate 1, G, shows the heads and necks of Little Club, C. I. 4066. This variety is always very severely rusted at University Farm, St. Paul. It sometimes is so severely injured by stem rust that, when heads are produced, only very small shriveled kernels are formed. The uredinia just below the rachis are very large and confluent. The epidermis breaks off as large scales, exposing masses of spores in a single large uredinium beneath.

STRUCTURE OF THE STEM

In order to study the structure of the stem, several wheat varieties were grown in adjacent rows. When the heads were in the soft-dough stage, stems were killed and cleared in aceto-alcohol. Sections 10μ thick were then cut in pith by means of a sliding razor. Hydrochloric acid-orcin, hydrochloric acid-phlorglucin, and analin-sulphate were used to differentiate the cellulose and pentosan structures. For permanent mounts for photomicrographs, sections were stained with Pianeze stain,

passed through acid alcohol, cleared in carbol-turpentine, passed through xylol and then mounted in balsam. Light staining with iodin clearly brought out the chlorophyll-bearing areas of the stem.

The wheat stem consists of a hollow cylinder, except in solid-stemmed varieties. (Pl. 2, A and B.) The epidermis, of course, consists of a single layer of cells, the inner walls of which are sometimes lignified. The number of stomata per unit area of surface differs in different varieties. Just beneath the epidermis is the chlorenchymatous collenchyma. This sometimes extends in an almost continuous band around the entire stem, although it usually is interrupted by strands of sclerenchyma. The collenchyma cells then are aggregated into isolated bundles, the size and number of which vary considerably in different varieties. The vascular bundles are arranged in a more or less definite ring in the thin-walled parenchymatous tissue of the fundamental ground tissue. Toward the inside is the pith. The bundles may be arranged in one or more rings; usually there are about 15 large bundles and a variable number of smaller bundles outside of the larger ones. A more or less continuous sheath of sclerenchymatous fibers extends between the different bundles and outside of them. Strands from this sheath often extend from the bundles through the collenchyma to the epidermis. It is particularly important to remember that the only chlorenchymatous tissue in the stem is the collenchyma. These extensions of the sclerenchymatous tissue interrupt the continuity of the collenchyma and break it up into bundles, the size of which depends upon the amount of schlerenchymatous tissue which develops.

This same general structure was shown by Sappin-Trouffy (*47, p. 103*) for oat stems and is figured for the sheath of wheat by Eriksson and Henning (*12, pl. 4, fig. 40 b*).

Photographs of sections of the upper peduncle or neck of four varieties of wheat—Kota, C. I. 5878; Little Club, C. I. 4066; Marquis, C. I. 3641; and Sonem emmer, C. I. 4402—are shown in Plate 2, C, D, E, and F. It is evident that the relative proportion of sclerenchyma to collenchyma is not the same in each variety. In Kota the sclerenchyma divides the collenchyma into distinct areas, while in Little Club the sclerenchyma is much less conspicuous and the collenchyma is practically continuous. In the Marquis stem there are somewhat more sclerenchyma fibers than in the Little Club stem and there also is a greater tendency for union of the collenchyma areas than in the stem of Kota. In the stem of Sonem emmer a large amount of sclerenchyma always is developed.

The morphological difference which may be expected in stems of different wheat varieties is illustrated in figure 1. The illustrations are actual tracings of permanently mounted sections prepared in the course of these experiments. In order to secure these tracings, the slides bearing the sections were placed on the stage of the microscope attachment of a projection lantern. The sections were then projected on white cardboard, placed 6 feet from the lantern. This greatly enlarged the sections, so that the details of structure could easily be traced with a pencil. The epidermis and fibrovascular bundles, together with all sclerenchyma, then were filled in with India ink, following which tracings of the different varieties were placed on a single large card and photographed. The interesting fact is the marked difference in the extent and distribution of the collenchyma areas in the different varieties. Figure 1, A, is made from a tracing of a section of Sonem emmer, C. I. 4402. In three years' observation this variety has shown less infection from both *P. triticina*

and *P. graminis* than any other wheat grown at University Farm, St. Paul. The collenchyma areas are extremely small and make up but little of the stem structure. The sclerenchyma areas are decidedly predominant in this variety and make up the major portion of the stem proper. The unevenness of the surface made by the bulging out of the

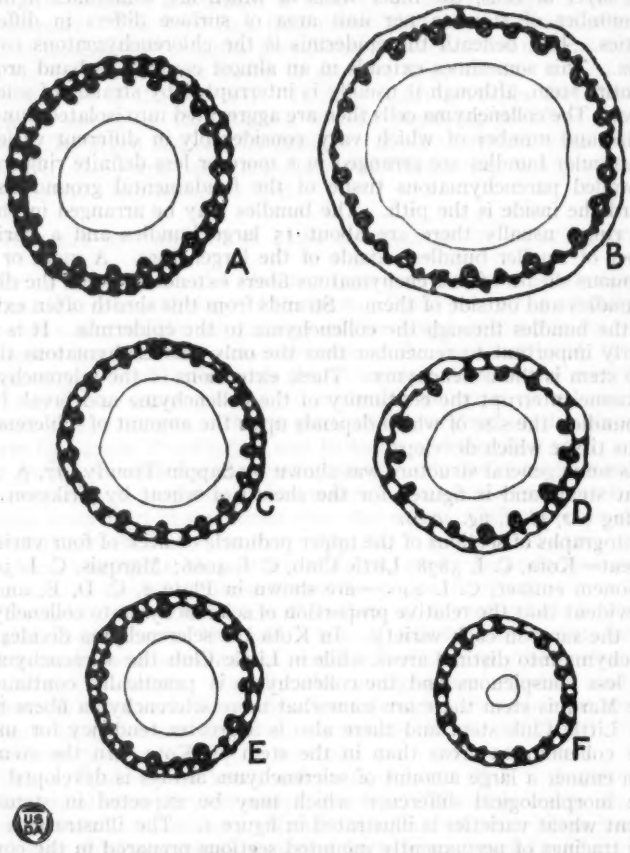

FIG. 1.—Tracings of transverse sections of the upper peduncle or neck of different wheat varieties. The sclerenchymatous tissues are represented by the black areas. Note the practically continuous band of collenchyma just under the epidermis in B; note also the relatively large collenchyma bundles in D. (× 25.)

A.—Sonem emmer, C. I. 4402.
B.—Little Club, C. I. 4066.
C.—Vernal (White Spring) emmer, C. I. 3686.
D.—Marquis, C. I. 3641.
E.—Kota, C. I. 5878.
F.—Einkorn, C. I. 2433.

sclerenchyma between the chlorophyll-bearing areas is very prominent. This condition is characteristic of all the emmer varieties studied, although the unevenness is particularly evident on Sonem emmer.

Figure 1, B, shows a tracing of a section of Little Club, C. I. 4066. This variety as infected by rust in the field, is shown in Plate 1, E.

Sections of its stem also are shown in Plate 2, D. In marked contrast to figure 1, A, the chlorophyll-bearing area is quite extensive and continuous. The sclerenchyma is confined to the regions between the fibrovascular bundles. The surface of the stem is smooth and even. Figure 1, C, is of Vernal (White Spring) emmer, C. I. 3686. This variety is similar to Sonem emmer in the extensive development of sclerenchymatous tissues. A tracing of a section of the stem of Marquis, C. I. 3641, is shown in figure 1, D. In this variety the collenchyma areas are smaller than similar tissues in Little Club, but, nevertheless, they are considerably larger than the collenchyma areas of the emmer varieties. Figure 1, E, is a tracing of Kota, C. I. 5878. A photograph of the variety is shown in Plate 1, F. Figure 1, F, shows a tracing of Einkorn, C. I. 2433, a variety of wheat possessing a stem of small diameter, compared with that of other varieties. All of the tracings shown in figure 1 have been traced on the same scale and show relative size.

It must be kept in mind that the diagrams shown illustrate the tendency of a variety to produce a certain type of structure and that certain fluctuations are to be expected in individuals. Even Little Club, when sectioned some distance from the rachis, shows the chlorophyll areas, beginning to be divided by sclerenchyma, but never to the extent shown in other varieties. Plate 1, F, shows this fact in that the pustules at a distance from the rachis are taking on a more elongated appearance. On the other hand, out of hundreds of sections made, never has a section of emmer shown any tendency of the collenchyma areas to be confluent. Considering the established fact that the development of the mycelium of *P. graminis* is practically limited to the collenchyma of the stem, it is not difficult to see that the different varieties studied will necessarily not show the same amount of stem-rust injury when subjected to a rust epidemic.

STEM STRUCTURE AS RELATED TO RUST DEVELOPMENT

Sappin-Trouffy (*47, p. 93*) states that the action of *P. graminis* on oat stems is quite restricted, as the mycelium is unable to extend except longitudinally, because it is bounded at the sides by a hemispherical sheath of sclerenchyma fiber. The same is true for the action of *P. graminis* on wheat stems. On the Kota stem the uredinia are linear each one being confined to a single collenchyma bundle. (Pl. 2, G.) In the neck of Little Club the mycelium is not limited by sclerenchyma strands but is free to extend laterally and therefore produces very large pustules. (Pl. 2, H.) Comparing these photographs with Plate 1, F and G, the importance of the sclerenchyma in relation to the size of the pustule is clear.

If the opportunities for infection are exceptionally favorable and a large number of individual infections result, even the varieties with extensive sclerenchyma may become severely rusted. Under such conditions more susceptible varieties, such as Little Club or Marquis would be seriously injured. It is quite possible, on the other hand, that when only a few infections take place, these latter varieties may be severely attacked, while the emmer varieties may escape comparatively unharmed.

The structure of the stem affects only the extent of the spread of the organism and its subsequent rupture of the epidermis. Resistance to stem rust must be considered as being due fundamentally to a complex physiological relationship. The results obtained by Stakman and Levine (*54*) show the difficulty in explaining actual resistance of wheat varieties

on morphologic grounds alone. Actual resistance is in its finality a matter of the individual cell. We may picture the collenchyma cells of the neck of Little Club wheat as so many easily rotted fruits in a large container where the fungus may spread quickly throughout the entire mass, infecting them all. The collenchyma cells in the stem of such a variety as Kota or Vernal (White Spring) emmer, when attacked by a biologic form to which it is susceptible, would be like the same fruit packed in small baskets, so that when one container was infected the fungus could not pass to the next. Thus a large part of the fruit would remain uninjured. On the other hand, if one conceives two kinds of fruit, one resistant and the other susceptible, both of them packed alike, the amount of actual injury sustained is placed on an entirely different basis. Of the two bases, the latter is a more fundamental type of resistance, and it is the type emphasized by Stakman and Levine in identifying biologic forms. Acme, C. I. 5284, a wheat variety reported by Stakman and Levine to be highly resistant to only 1 biologic form in 37, suffers very little from rust attacks in the field at University Farm, St. Paul. A section of the neck of this variety is shown in Plate 2, I. It has a strong tendency to produce sclerenchyma fibers between the epidermis and the collenchyma, a characteristic not observed in any other variety studied. The amount of sclerenchyma shown in Plate 2, I, is unusually large for Acme.

The diameter of the stem may have some effect on the amount of injury caused by rust. Other factors being equal, the diameter of the stem alone would tend to determine the resistance to rupture, etc. The smaller diameter gives the greater surface curvature with the greater resistance to rupture. That this resistance to rupture may be of some significance is shown by the fact that the mycelium may develop within the collenchyma areas of certain small-stemmed varieties with abundant sclerenchyma, and yet produce no uredinia. This condition is quite common in the emmers. Within a given variety, any factors which would increase the size of the stem or the proportion of collenchyma to the sclerenchyma would also tend to increase the actual injury resulting from a rust attack. The many statements by investigators concerning the heavier rust infections due to manuring with nitrogenous fertilizers may be in some way partly connected with this fact.

RELATION OF NUTRIENT SALTS TO THE DEVELOPMENT OF STEM RUST

A series of sand cultures was set up for the purpose of studying the relation of certain nutrient salts to the morphology and physiology of the wheat plant, and the possible indirect influence of these salts on the development of stem rust.

EXPERIMENTAL PROCEDURE

A clean, white quartz sand was secured from the division of soils of the Minnesota Agricultural Experiment Station. This was best Ottawa quartz and contained so little nutrient material as to be unable to support growth. All the salts used in making up the nutrient solutions were chemically pure but were not recrystallized.

In setting up this series of cultures two liters of sand were placed in each crock, to which was added 1,900 cc. of distilled water containing the entire amount of the salts used. A constant water table was maintained by adding distilled water. A water gauge was inserted in the hole at the bottom of the crock and paraffined in to insure a ready means of observing the water table for each pot. In addition to the water gauge from the bottom of the crock, an inverted thistle tube was placed in the center, so that the bell of the tube, covered with cheesecloth, was 2 inches from the bottom. This furnished good aeration and permitted excellent root development.

Two series were set up on different dates, January 29, 1922, and June 14, 1922. Haynes Bluestem wheat, C. I. 2874, from the same seed lot, was sown in each series. Tables VI and VII give the salt combinations used.

TABLE VI.—*Salts used in series of sand cultures started January 29, 1922, to determine the relation of nutrient salts to development of stem rust*

Series No.	Salt.	Grams per liter.	Gram molecules per liter.
101	$Ca(NO_3)_2$	1.279	0.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
102	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	KNO_3	1.729	.0171
103	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$MgHPO_4$	2.982	.0171
104	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	K_2SO_4	2.980	.0171
105	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$NaNO_3$	1.454	.0171
106	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$CaHPO_4$	2.943	.0171
107	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030

TABLE VII.—*Salt combinations used in series of sand cultures started on June 14, 1922, to determine the relation of nutrient salts to development of stem rust*

Series No.	Salt.	Grams per liter.	Gram molecules per liter.
121	$Ca(NO_3)_2$	1.279	0.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$FeSO_4$	0.020	.0001
122	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$FeSO_4$	0.020	.0001
	K_2HPO_4	1.882	.0136
123	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$FeSO_4$	0.020	.0001
	KNO_3	0.864	.0015
124	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$FeSO_4$	0.020	.0001
	KNO_3	1.729	.0171
125	$Ca(NO_3)_2$	0.128	.0008
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$FeSO_4$	0.020	.0001
	$CaHPO_4$	2.943	.0171
126	$Ca(NO_3)_2$	1.279	.0078
	$CaHPO_4$	0.294	.0017
	$MgSO_4$	0.361	.0030
	KNO_3	1.729	.0171
	$FeSO_4$	0.020	.0001
127	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$FeSO_4$	0.300	.0007
128	$Ca(NO_3)_2$	1.279	.0078
	KH_2PO_4	1.471	.0108
	$MgSO_4$	0.361	.0030
	$AlCl_3$	1.895	.0171

When the seedlings in the series of January 29 were two months old, in place of the regular watering with distilled water, a quantity of the particular salt being tested for each series was added to each crock in the series as follows:

Series No.	Salt added Apr. 1.
101	250 cc. distilled water, no salt.
102	250 cc. distilled water, containing 0.432 gm. KNO_3.
103	250 cc. distilled water, containing 0.745 gm. $MgHPO_4$.
104	250 cc. distilled water, containing 0.745 gm. K_2SO_4.
105	250 cc. distilled water, containing 0.363 gm. $NaNO_3$.
106	250 cc. distilled water, containing 0.736 gm. $CaHPO_4$.

The plants of this series were then inoculated on April 1, 1922, with urediniospores of biologic Form XXXIII. This form, as shown by Stakman and Levine (*54*), produces a type "2" infection on Haynes Bluestem. It was hoped that a form which produced a type 2 infection would be more sensitive to changes of the host than a form to which the host is very susceptible or very resistant. The different pots of the same salt combinations were not placed together in the incubation chambers but were scattered at random so as to prevent any two pots with the same salt combination from being together. The plants were so heavily inoculated by dusting with urediniospores that the spore mass could be seen easily on the leaves. The cloth chambers were then covered with wet cheesecloth, an atomizer was inserted through slits along the sides near the top and a fine mist spray was forced into the chamber. The air remained saturated throughout the 48-hour incubation period, following which the plants were placed on the greenhouse bench for 14 days. No observations were made on the stomatal movements, although this would have been desirable.

The series of June 14 was exactly like the previous one except that no additional salt solution was added at the time of inoculation. The plants were inoculated on July 19, when about 5 weeks old. They, therefore, were younger than those of the first series, but they were fully as well developed, owing to the difference in available sunlight.

RESULTS OF INOCULATION WITH BIOLOGIC FORM XXXIII

The degree of rust infection on the seedlings is given in Tables VIII and IX.

TABLE VIII.—*The effect of different nutrients on the growth of Haynes Bluestem wheat plants in sand cultures started January 29, 1922, and on the development of stem rust caused by biologic Form XXXIII used as inoculum on April 1, 1922*[a]

Series No.	Changes in basic nutrient solution.	Percentage of plants infected.	Severity of infection.	Character of growth.
101	None (control)....	None.....	.....	Good.
102	KNO_3 added.....	85.....	Medium ...	Excessive tillering.
103	$MgHPO_4$ added ..	None.....	.....	Medium.
104	K_2SO_4 added.....	1 uredinium on 1 plant.	Medium ...	Do.
105	$NaNO_3$ added	70.....	...do.....	Fair; much tillering.
106	$CaHPO_4$ added...	None.....	.....	Medium.

[a] These observations were made by Dr. E. C. Stakman and M. N. Levine.

TABLE IX.—*The effect of different nutrients in sand cultures started June 14, 1922, on the growth of Haynes Bluestem wheat plants and on the development of stem rust caused by biologic Form XXXIII, used as inoculum on July 19, 1922*

Series No.	Changes in basic nutrient solution.	Percentage of plants infected.	Severity of infection.	Character of growth.
121	None (control)	60	Medium	Good.
122	Phosphate increased by adding K_2HPO_4.	20	do	Medium.
123	Nitrate increased by adding KNO_3	80	Severe	Excessive tillering.
124	Nitrate further increased by adding twice as much KNO_3 as in 123.	90	do	Do.
125	Nitrate decreased to one-tenth; phosphate increased by adding $CaHPO_4$.	0	No infection.	Fair; leaves narrow; sandy.
126	Phosphate decreased to one-fifth, using $CaHPO_4$; nitrate increased by adding KNO_3.	20	Medium	Poor.
127	$FeSO_4$ increased by adding 15 times as much.	50	do	Good.
128	$FeSO_4$ omitted; $AlCl_3$ added	(a)		Very poor; plants killed by root-rotting fungi.

[a] Not inoculated.

It will be seen from Table VIII that there was no rust on any plants except those grown in sand to which considerable nitrogen had been added. A single exception was one uredinium which developed on a plant in the potassium sulphate series. Table IX shows that in the second series the rust developed more abundantly on the plants fertilized with a large amount of nitrogen. However, in this series the rust failed to develop only on plants receiving a very poor balance of nitrogen and phosphorus.

The plants in the pots receiving excess nitrate tillered more than those in the other pots and developed leaves of a different texture. The lower epidermis was removed from a leaf of a plant receiving an excess of potassium nitrate and compared with that of a leaf from a plant in soil deficient in nitrate. (See Pl. 9.) The epidermis from the plants supplied with abundant nitrogen is smooth, with few hairs and but little bloom, while on plants with a poor balance, or a lack of nitrogen, the leaves are rough to the touch, more hairs are present, the bloom can readily be seen, and there are fewer stomata.

The relation of nutrient salts to the type of cell wall and general character of the epidermis has been briefly mentioned in the historical review. Petermann (*40, p. 15–16*) states that the cell walls are much stronger in wheat plants on a low plane of nutrition, while manures produced rusted plants, owing to the fact that the haustoria could penetrate more easily. Palladin (*38*) asserts that cereals grown in soils deficient in silicic acid often rust so severely that it is difficult to prevent their complete destruction. Russell (*46*) states that nitrogenous manures produce plants with thin walls, easily attacked by fungous hyphae. Comes (*9*) also maintains that organic fertilizers increase the tenderness of the plant and favor fungous attack.

Bolley (*7*), Farrer (*13*), and Cobb (*8*) state that wheat varieties with stiff, upright growth and narrow leaves are less susceptible to *P. triticina* than are those with broader leaves and less erect habit of growth. Here, again, the germ tubes may have been prevented from entering on account of the failure of the stomata to open. The morphological characters pointed out by Cobb and others can be produced in any variety when it is grown under extreme conditions of soil fertility.

Harter (*20*) found that wheat plants grown in soils to which had been added from 0.7 to 1.4 per cent sodium chlorid developed a heavy bloom on the leaf surface, thickened cuticle, and epidermal cells of reduced size. This condition may not have resulted from any physiologic action of the salt itself within the plant tissues. These xerophytic characters, together with decreased transpiration, probably are due to the influence of the salt on the entrance of other soil nutrients; and, consequently, only a very poorly balanced nutrition is available to the plants.

It appears from Table VIII that excessive nitrogen rendered plants more subject to attack by stem rust. After inoculation these plants were kept in a saturated atmosphere and consequently the leaves were covered with a film of moisture. Iljin (*26*) concludes from his experiments on the transpiration of wet leaves that immersion in water in the dark has the tendency to cause closure of the stomata. However, if potassium nitrate is present in the water, the stomata open.

Many investigators have shown that under certain conditions the amount of nitrate in the leaves of a plant supplied with an excess of this salt may be proportionately higher than in normal plants. Whether this condition can be associated with the findings of Iljin has not been determined.

Rust appeared only on plants in the nitrate series. It is possible that the environmental conditions during the incubation period of the series of January 29 were such that the stomata of all the plants except those growing in soil with high nitrogen content remained closed. This supposition is supported by the fact that, although the spores germinated profusely and abundant appressoria were formed on all plants, no flecks appeared on any of the plants which received no nitrogen, indicating that the lack of infection more likely was due to failure of germ tubes to enter than to any induced protoplasmic resistance. This explanation is partly confirmed also by the behavior of the plants in the second series. Here all plants became infected except those in series 125, which were grown in soil deficient in nitrogen. These plants were maturing early, the leaves were narrow, the stems upright, stiff, and provided with abundant bloom. The leaves, particularly, were rough and dry to the touch.

Morphology of Host as Influenced by Nutrient Salts

In order to compare the morphology of plants of Haynes Bluestem wheat grown under different conditions of nutrition, sections of the leaves were cleared and stained with Pianeze stain. Four such sections are shown in Plate 2, K, L, M, and N. Here, L and N show typical sections of a leaf from a plant grown in abundant nitrogen, not balanced by other nutrients. The epidermis is composed of very large, thin-walled, noncutinized cells. There is very little sclerenchyma, and the fibers do not extend from one leaf surface to the other. The intercellular spaces are very large, the leaf being porous and succulent. Plate 2, K

and M, shows sections of a leaf from a plant grown in a culture deficient in nitrogen but containing an excess of phosphates. The contrast with Plate 2, L, is very marked. The epidermis, particularly on the lower surface, is composed of small, highly cutinized, thick-walled cells. Associated with the fibrovascular bundles is a relatively large amount of sclerenchyma, which extends from surface to surface. The chlorenchyma is compact and the intercellular spaces are small. The leaf is compact and firm. These sections were made from leaves of the same age, of the same wheat variety, and grown under the same conditions, the difference being only in the nutrient salts which the plants received.

Kraus and Kraybill (*28*) have shown that feebly vegetative tomato plants, grown in soil with a small amount of nitrogen, contain practically no nitrate nitrogen and but little total nitrogen. In these plants the dry matter and free reducing substance are comparatively high. The bast fibers and xylem tissues are greatly increased. On the other hand, plants growing under favorable vegetative conditions have higher total nitrogen and nitrate nitrogen and lower free reducing substance and dry matter. The bast and xylem tissues are comparatively greatly reduced.

Such studies suggest the possible function of potassium and sodium nitrate in the physiology of the plant. These salts appear to be concerned chiefly with carbohydrate utilization. If carbohydrates are not normally utilized there would tend to be an increase in the production of cell wall, crude fiber, and pentosans. The deficiency of nitrogen in the plants reported in these studies appears to result in thickened cell walls, as shown in Plate 1, D, and in an increase of fibers, as shown in Plate 2, L and M.

Plate 2, J, illustrates the typical restriction of rust development by the morphology of a wheat leaf. The sclerenchyma cells extend from the upper epidermis to the lower epidermis and permit the development of mycelium in only the longitudinal direction. Plowright (*41*) states that there is a tendency for the mycelium at the base of uredinia to spread in a centrifugal manner, but that many causes operate to prevent this, the chief being the lack of uniformity in the tissues of the host plant; and in a leaf with strongly marked venation this tends to exert a directive influence upon its extension. Eriksson and Henning (*12, pl. 10, fig. 110*) show a uredinium of *P. dispersa* on the seedling leaf of Bromus sp., and call attention to the concentric nature of the secondary uredinia, showing that the mycelium has spread in all directions from the original point of infection. The same condition occurs also in the wheat seedling infected with *P. graminis*. However, as figure 112 of the same plate shows, there is no such secondary infection on the older leaves. The sclerenchyma in the seedling leaf does not extend from epidermis to epidermis and, consequently, does not restrict the growth of the mycelium but permits it to grow in all directions. In the older leaves so many bundles of fibers have developed that the growth of the mycelium is restricted to the areas between the fibrovascular bundles.

Referring again to Plate 2, K, L, M, and N, it is possible to understand the influence of a nitrogenous fertilizer in increasing the size of the uredenia on the host plant. If the succulent leaf, Plate 2, L, becomes infected, the growth of the fungus would probably be rapid and extensive because there are no sclerenchyma fibers between the bundles and the epidermis to prevent its growth. The uredinia therefore would be large. But the growth of the distributive hyphae in such a leaf as that shown in Plate 2, K, would be restricted by the sclerenchyma fibers. Small

uredinia would be produced, because each one would be restricted to a single collenchyma bundle. There is likely to be relatively little sclerenchyma in leaves of plants which have been fertilized with an unbalanced excess of nitrogen; and the uredinia which develop on them therefore may be larger than those on plants which have been fertilized normally. While the actual protoplasmic resistance of the plants may not have been changed, there may be a greater total area of tissues in which the rust mycelium can grow.

Furthermore, heavy nitrogenous fertilization promotes rank growth and dense stands of wheat. Moisture, therefore, is retained and the opportunities for spore germination are increased. An excess of nitrogen also seems to increase the transpiration of the plants, causing the stomata to open and thus facilitate the entrance of the germ tubes.

The balance of nutrients seems to be much more important than the total amount. The results of field experiments on the effect of fertilizers on rust development are therefore significant only if the soil type is definitely known.

THE RELATION OF PHYSICOCHEMICAL PROPERTIES OF THE PLANT SAP TO RUST RESISTANCE

As some varieties of wheat are completely susceptible to certain biologic forms of *P. graminis tritici* and are immune from others, it is obvious that the sole basis of resistance can not be morphological characters. It is a well established fact, for instance, that Kanred is completely susceptible to some biologic forms and it is so highly resistant to others that not even flecks are developed on plants inoculated artificially. It is known, also, that the mycelium of the biologic forms to which Kanred is resistant can not develop extensively within the tissues. Therefore, there is a real protoplasmic or physiologic resistance. The exact nature of this resistance has never been determined. Hurd (*24*) studied the possible relation of hydrogen-ion concentration of wheat varieties to their resistance to rust and other pathogenic fungi, and concluded that hydrogen-ion concentration probably had little effect on resistance. In fact, the concentration varied more in the same variety grown under different conditions than it did in different varieties.

The osmotic concentration of the sap of a host plant probably exerts considerable influence on the ability of the rust fungus to absorb nutriment from it. The total solids indicate the amount of moisture in the plant tissues and therefore to a certain extent are correlated with the succulence of the plant. It is possible, therefore, that differences in total solids might affect the development of rust. The importance of available carbohydrates in the nutrition of rust fungi has been shown clearly by Mains (*33*). Henning (*22*) and Kirchner (*27*) were of the opinion that the content of reducing sugars was lower in rust-susceptible than in resistant varieties. Eckerson (*11*) has shown that the carbon substances vary in relative amount in the plant sap at different stages of development of the wheat plant.

The writer made attempts to ascertain whether there were differences in the physicochemical properties of different varieties great enough and sufficiently consistent to account for differences in their resistance to rust. Particular attention was paid to the determination of the depression of the freezing point, total solids, average molecular weight, hydrogen-ion concentration, and sugar content. The following methods were

used: Seedlings of the different varieties were frozen in carbon dioxid and pulverized while still frozen. The powdered plant tissue was thawed at room temperature and the sap then extracted by pressure. Freezing-point depression was determined by means of a Beckmann thermometer. Total solids and average molecular weight of solutes were determined by the refractometric method described by Gortner and Hoffman (*18*). A Leeds and Northrup type (K) potentiometer was used in securing the hydrogen-ion values. The results of these determinations are given in Table X. For analysis of sugars present in the sap, seedlings were grown under continuous light.[4] Samples of 25 gm. each, green weight, were extracted in methyl alcohol, and total and reducing sugars determined by the picric acid method described by Rose (*45*). Dry weight was determined from separate samples dried at 65° in vacuum. The results of sugar determinations are given in Table XI.

TABLE X.—*Physicochemical properties of the sap of six wheat varieties*

Wheat variety.	Freezing-point depression.	Refractometric solids.	Average molecular weight of solutes.	P_H.	Relative susceptibility.[a]
	°C.				
Khapli, C. I. 4013	0.95	8.50	173	5.984	2.2
Mindum, C. I. 5296	0.98	.....	.....	6.194	41.2
Kota, C. I. 5878	0.91	7.55	177	6.062	40.1
Kanred, C. I. 5146	1.08	8.40	159	5.890	32.8
Marquis, C. I. 3641	1.02	7.00	138	5.973	60.8
Little Club, C. I. 4066	0.82	6.46	155	5.899	88.4

[a] 100=completely susceptible. Unpublished computations of E. C. Stakman and M. N. Levine on the basis of reaction of wheat varieties to all known biologic forms of *P. graminis tritici*. Little Club, while susceptible to all biologic forms, does not always produce the most virulent type of infection.

TABLE XI.—*Determination of sugars in sap of six wheat varieties*

Wheat variety.	Reducing.		As sucrose.		Total.	
	Percentage of green weight.	Percentage of dry weight.	Percentage of green weight.	Percentage of dry weight.	Percentage of green weight.	Percentage of dry weight.
Khapli, C. I. 4013	0.6856	7.4072	0.1420	1.5176	0.8276	8.9248
Mindum, C. I. 5296	.6857	7.3896	.2376	2.5704	.9242	9.9600
Kota, C. I. 5878	.7680	8.3296	.0809	0.5312	.8489	8.8602
Kanred, C. I. 5146	.8531	8.9392	.0000	0.0000	.8531	8.9392
Marquis, C. I. 3641	.7515	8.0992	.0000	0.0000	.7515	8.099
Little Club, C. I. 4066	.7315	8.6544	.0000	0.0000	.7315	8.654

Table X shows that there are some differences in the physicochemical properties of different wheat varieties. There is a smaller percentage of solid matter in the sap of Little Club than in that of the other varieties. This is indicated both by the freezing-point determination and refractometric methods. There appears to be no correlation, however, between

[4] The plants were grown in one of the constant-light rooms described by Harvey (*21*).

the amount of solids in the sap and rust resistance. The hydrogen-ion concentrations of the different varieties differed but little, an observation agreeing with that of Hurd (*24*). The data presented in Table X show that there is no consistent correlation of any of the sap properties of different varieties and their relative degree of resistance to biologic forms of *Puccinia graminis tritici*.

The data given in Table XI indicate that, under the conditions of the experiment, varieties show a difference in the amount of both reducing sugar and total sugars. No sucrose was present in Little Club, Marquis, and Kanred, all of their sugars analyzing as reducing sugar. These varieties also contained higher concentrations of reducing sugars than did Khapli and Mindum. In Khapli and Mindum 17 and 25.8 per cent, respectively, of the total sugar was sucrose. Khapli is resistant to all of the biologic forms described by Stakman and Levine; Mindum is resistant to some and susceptible to others. From the data presented in Table XI, we are not justified in drawing any definite conclusions as to the relation of sugar content to rust development. It can be pointed out that wheat varieties do differ considerably in their sugar content and that detailed investigations may give significant information both on the general nature of the relation of rust development to sugar content of the host sap and to the difference in behavior of biologic forms. Such an analysis is of little value, however, without a complete analysis of the carbon metabolism, changes in plant acids, etc.

The difficulty in attempting to explain the nature of resistance to stem rust on the basis of specific physicochemical properties of the sap of a variety is because of the difference in behavior of a particular variety to a number of biologic forms of *Puccinia graminis tritici*. Khapli is quite resistant to all of the biologic forms studied by Stakman and Levine and it is conceivable, though not proved, that this resistance may be due to certain physicochemical properties.

Little investigation has been made to determine physiological differences between biologic forms of *P. graminis tritici*. The writer previously has reported that a distinct physiological difference can be demonstrated in biologic forms apart from the differential hosts used in identifying them. It was shown (*25*) that urediniospores of two biologic forms germinate differently under different conditions of temperature and hydrogen-ion concentration. Furthermore, the urediniospores of a biologic form which attacks only a few differential hosts could not germinate well under such a wide range of environmental conditions studied as could those of a biologic form which can attack many hosts. That is to say, the potentiality or possibility for germ-tube development is greater in the biologic form with a wide host range. An organism restricted in its activity to a narrow temperature range might possibly also have a narrow range of adjustment to other environmental variables. In seeking for the significance of this fact in relation to rust resistance, however, we must turn to the physiology of the host.

We know that there are variations in the morphologic and physiologic characters of varieties within a species. Vavilov (*56*) has concluded that these variations may occur as homologous series within related taxonomic groups. It is probable, therefore, that there also are physiologic variations. Since resistance is in part physiological in nature, or expresses itself as such, we see that different wheat varieties present to a rust organism a range of physiologic reaction with which it must compete successfully if it is able to infect. It would appear, then, that a

biologic form which has the potential ability to adjust itself to a wide range of conditions or reactions might be able to maintain itself in a greater number of host varieties than would a biologic form with limited range of tolerance to certain physiologic reactions.

In the same way there may be corresponding differences in the physiology of biologic forms of *P. graminis*, and this may account even for the existence of biologic forms. Then it would naturally follow that forms differ in ability to adjust themselves to physiologic environment.

It is reasonable to expect that all biologic phenomena are ultimately to be explained on a physicochemical basis. The action of poisons and toxins are not exceptions. A conception of biologic forms based on physiological studies, as has just been indicated, would be quite in keeping, therefore, with the accepted ideas of fundamental resistance.

GENERAL CONCLUSIONS

It is obvious that the basis for resistance of wheat varieties to biologic forms of *P. graminis tritici* must be either morphological or physiological. For a long time it was supposed that the basis of this resistance might be morphological. The work of Ward, however, indicated that external morphological characters probably were of minor importance in determining resistance or susceptibility of plants to the attack of rust fungi. More recently still, however, the work of Allen and others, together with extensive field observations, has called attention to the fact that morphological characters might play some part in determining resistance. It has been suggested that the morphology of the plant might affect resistance by preventing the entrance of germ tubes.

It seems reasonable to suppose that certain morphologic characters might possibly interfere with the entrance of germ tubes. As the germ tubes of the urediniospores always enter the wheat plant through stomata, it would seem especially reasonable to suppose that the number, distribution, size, and location of the stomata might be quite important in determining how many germ tubes could enter the plant. Furthermore, we may assume that the number of hairs on the plant surface may have some effect on the growth of the germ tubes, as an abundance of hairs easily may block the growth of the tubes. Extensive observations were made on the number of hairs and the number and size of the stomata on susceptible and resistant varieties of wheat. While the number of hairs in general was greater on the more resistant varieties than on the more susceptible ones, it seemed evident that the number of hairs scarcely could be a determining factor in the entrance of the germ tubes. The number of stomata, as well as the size of the stomatal aperture, differs for different varieties. However, some of the most resistant hosts have stomata with large apertures, while some very susceptible ones have stomata with small apertures. It seems probable that the stomatal aperture of all varieties is sufficiently large to permit the entrance of urediniospore germ tubes. It seems much more likely that the character of the stomatal movements, which may differ in different varieties, would have more effect upon entrance. The size of the stomatal opening scarcely could account for the resistance of certain varieties to certain biologic forms, because some varieties are immune from certain biologic forms and completely susceptible to others. It never has been demonstrated that the size of germ tubes of the different biologic forms differs sufficiently to make an explanation of resistance on this basis of entrance

seem reasonable. It is quite true that appressoria may be produced fairly abundantly on the surface of certain plants, without any subsequent development of the germ tubes. The failure of the fungus to enter, even after appressoria have been formed, probably must be explained on the basis of the absence of the necessary stimulus or possibly by the failure of stomata to remain open sufficiently long to enable the rust fungus to enter.

There can be no question whatever but that there is a real physiologic or protoplasmic resistance. For instance, Khapli is resistant to all biologic forms of *P. graminis tritici*, though flecks practically always develop after inoculation. Naturally, therefore, the fungus must have entered the host tissue. It has been demonstrated beyond doubt that the cells of many resistant hosts are hypersensitive to the attacks of the fungus (*2*), (*34*), (*51*). They are killed very quickly after the fungus comes in contact with them, and the hyphae of the invading fungus then die.

It has been claimed that the physiology of the host plant can be changed rapidly and profoundly enough by altering environmental conditions to cause great variations in the protoplasmic resistance of the host to *P. graminis*. While this never has been demonstrated, certain observed facts seem to substantiate the claim. It frequently has been observed that the resistance of a variety apparently may change when conditions are altered. It also has been shown that young plants sometimes are more susceptible than older ones. For instance, Stakman and Piemeisel (*55*) have observed that young plants of *Agropyron smithii* can be infected easily as a result of artificial inoculations. It is much more difficult, however, to infect older plants. It also has been observed that certain varieties of wheat, such as Kota and Acme, are susceptible to a considerable number of biologic forms in the greenhouse but that they seem to be fairly resistant in the field. This fact must be due either to physiologic or morphologic changes in the host.

Extensive studies of the morphology of different varieties of wheat were made in order to ascertain whether there were any differences sufficiently great to account for the differences in rust resistance. It was found that the amount of sclerenchymatous tissue in the stem of Kota, Acme, and certain other varieties is very large in proportion to the amount of the chlorenchymatous collenchyma. As the rust can live practically only in the chlorenchyma, and as the only tissue in the stem which contains chlorophyll is the collenchyma, it is clear that the amount of collenchyma would determine to a very considerable extent the amount of development which is possible for the rust fungus. In certain resistant varieties the sclerenchymatous tissues have developed to such an extent as to decrease considerably the amount of space in which the rust can grow. Sclerenchymatous strands extend from the vascular bundles to the epidermis and a complete sclerenchyma sheath extends around the stem just outside the vascular bundles. The collenchyma bundles, therefore, are small and completely separated from each other. They are surrounded on three sides by thick-walled, lignified, sclerenchymatous fibers, through which the rust hyphae can not grow and from which they can not obtain nourishment. The size of the uredinia, therefore, is limited by the size of the collenchyma bundles. It is impossible for the rust to spread for any distance tangentially or radially. It can spread only longitudinally and this probably accounts for the fact that long,

narrow uredinia often are formed on varieties which appear to be generally resistant in the field.

The morphological structure of a stem can be changed by environmental conditions. It has been shown that the ratio of sclerenchymatous tissue to collenchymatous tissue can be reduced by unbalanced nitrogen nutrition of the plant. The effect of large amounts of nitrogenous fertilizers, therefore, especially when their effect is not counterbalanced by the addition of other fertilizers, probably is to increase the area in which the rust fungus can grow. In this way, fertilizers appear to sensitize plants to infection. We should not overlook the fact, however, that the fundamental protoplasmic resistance of the plant probably has not been changed. The individual cells probably are just as susceptible in plants fertilized with nitrogen as they are in those which have not been so fertilized, but the number of cells in which the rust can develop is greater. Conversely, phosphates and potassium fertilizers may cause an increase in the amount of sclerenchymatous tissues and thus limit the area in which the rust mycelium can grow.

It is quite likely also that the reason why seedlings sometimes seem to be more susceptible than older plants is that the morphology of the seedlings is somewhat different from that of the older plants. It has been observed frequently that varieties which are susceptible in the seedling stage behave in a peculiar manner in the field. Either very few uredinia are produced on the plants in the field or they remain small. The explanation for this fact probably is that the amount of sclerenchymatous tissue in proportion to the other tissues is greater in the stems of older plants than in the leaves of seedlings. While the basic resistance, therefore, of the plant is not different from what it was when the plant was young, still the area in which the rust can develop is limited mechanically.

No satisfactory explanation ever has been given for the basic or protoplasmic resistance of wheat varieties. It seems quite obvious that this must be due to physicochemical relations between the host and the pathogene. As the pathogene can not be grown in artificial culture media, a study of the problem is somewhat difficult. The most promising method of attack would seem to be to ascertain whether there are consistent differences in the physicochemical properties of different varieties. Determinations therefore were made of hydrogen-ion concentration, sugar content, etc. None of the observed differences seemed to be consistent or great enough to account for the differences in resistance. While it may be significant that the sugar concentrations in different varieties varied somewhat, too much importance should not be attached to these results. A study also was made of the reaction of spores of different biologic forms to certain physicochemical factors. It was found that the spore germination of two biologic forms was affected differently by hydrogen-ion concentration and by temperature. The form with the widest host range was able to withstand a wider range of variation than was the form with the narrower host range. This fact probably is of some significance, but just how much it is impossible to say.

While the question of the fundamental nature of resistance of wheat varieties to *P. graminis* was not completely solved, it at least has been shown that many of the rather puzzling and conflicting observations on apparent change of resistance can be explained easily on the basis of morphologic changes which occur within the host. It should be kept clearly in mind, however, that this is not a change in the fundamental

resistance but only a change in the opportunities of the rust to develop, due to a spatial limitation of the growth of the organism.

SUMMARY

1. In addition to fundamental protoplasmic resistance, wheat varieties may possess other means of defense against *Puccinia graminis*.

2. The number of leaf hairs and the size and number of the stomata can not be considered important in influencing the entrance of the germ tubes. When there is only a small amount of inoculum, the large number of hairs on some varieties may prevent some of the germ tubes from reaching the stomata and growing through the stomatal slits.

3. Stomatal movements may have some influence on the entrance of germ tubes. The stomatal movements of different varieties of wheat apparently are affected differently by environmental conditions.

4. The mycelium of *P. graminis* within the host is limited almost entirely to chlorenchymatous tissue. As the only important chlorenchymatous tissue of the stem is the collenchyma, the rust mycelium can grow only in this tissue. In some varieties of wheat there is such a large amount of sclerenchyma that the band of collenchymatous tissue is broken up into small bundles. The extent of mycelial development, therefore, is limited to these relatively small areas.

5. The amount of sclerenchyma is not the same in the stems of different varieties. In some there is a very large amount and in others relatively little. The amount of collenchyma is approximately inversely proportional to the amount of sclerenchyma. In those varieties, therefore, in which there is a large amount of collenchyma, large uredinia are likely to be produced, while in those varieties in which the collenchyma bundles are small, the uredinia are likely to be narrowly linear. Varieties in which there is a great deal of sclerenchyma are likely to be injured less by rust, as there is a mechanical limitation to the spread of the mycelium.

6. The relative proportion of sclerenchyma to collenchyma in a given variety may be altered by the use of fertilizers.

7. Excessive fertilization with nitrogen has a tendency to decrease the amount of sclerenchyma in proportion to the amount of collenchyma. For this reason, plants heavily fertilized with nitrogen may be more severely injured by rust than those which have not been so fertilized.

8. The fact that the seedlings of some varieties appear to be more susceptible to certain biologic forms of *P. graminis* than are the older plants can be explained by differences in morphology between the seedlings and the mature plants. There is a greater amount of sclerenchyma in the mature plants than in the seedlings and this constitutes a mechanical restriction on the growth of the mycelium.

9. There are differences in the physicochemical properties of the sap of different wheat varieties. It has been impossible, however, to make a definite correlation between these properties and rust resistance.

10. The differences in the reaction of wheat varieties to different biologic forms of *P. graminis tritici* appear to be due entirely to physiologic causes.

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PLATE 1

A.—Strip from epidermis of stem of Little Club wheat, C. I. 4066, showing relative number of stomata per unit area.

B.—Strip from epidermis of stem of Marquis wheat, C. I. 3641, showing relative number of stomata per unit area.

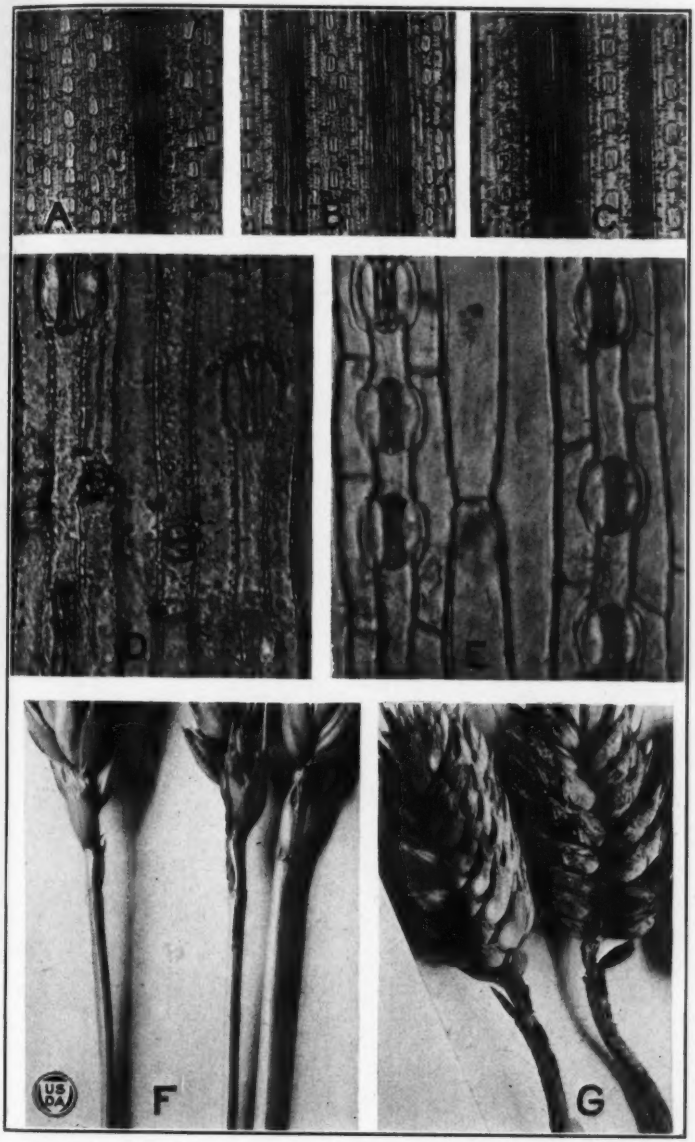
C.—Strip from epidermis of stem of Kota wheat, C. I. 5878, showing relative number of stomata per unit area. All ×75.

D.—Typical epidermis from plant of Haynes Bluestem wheat grown in nitrogen-deficient culture.

E.—Typical epidermis from plant of the same variety grown in culture supplied with excess nitrogen.

F.—Portions of heads and peduncles of Kota wheat, C. I. 5878, showing solitary linear uredinia on peduncle.

G.—Heads and peduncles of Little Club wheat, C. I. 4066, showing confluent, oblong uredinia.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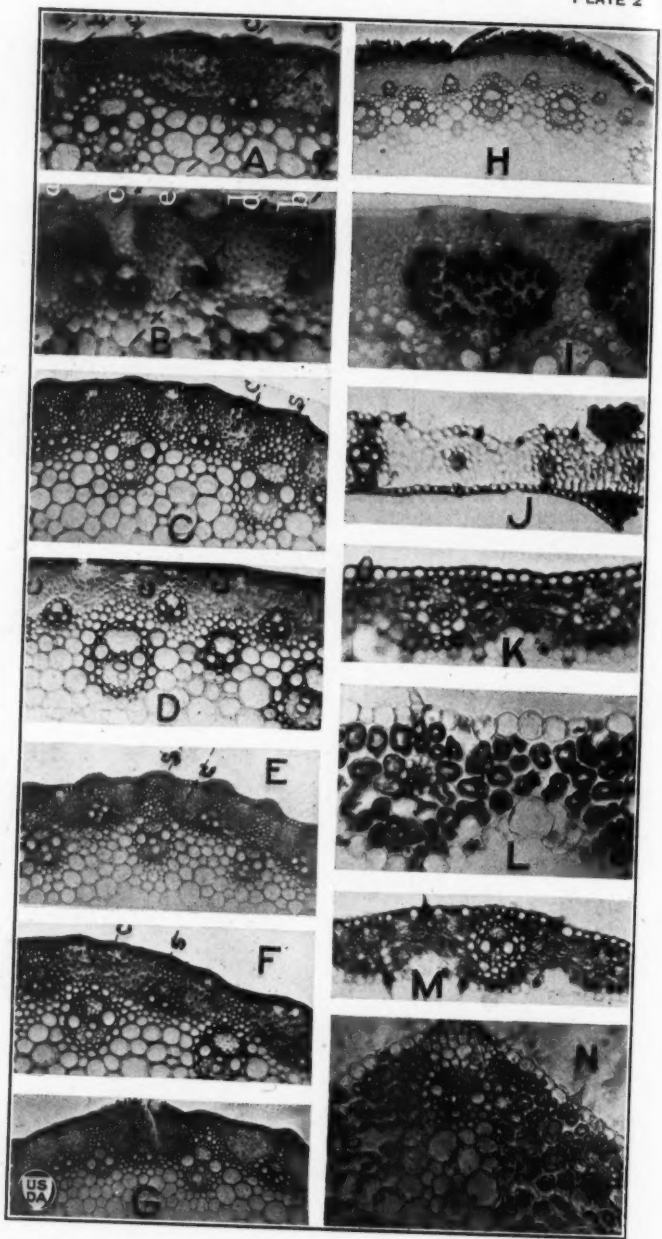
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PLATE 2

A.—Section of wheat stem showing a moderate amount of sclerenchyma fiber development. The tissues are as follows: (*a*) Epidermis, (*b*) collenchyma, (*c*) sclerenchyma fibers (×120), (*d*) thin-walled parenchyma, (*e*) fibrovascular bundles.

B.—Section of wheat stem showing a relatively small amount of sclerenchyma development in proportion to collenchyma. The tissues are lettered as in A.

C.—Cross section of upper part of peduncle of Kota wheat, C. I. 5878. The collenchyma (*c*) is restricted to small unit areas separated by the sclerenchymatous fibers (*s*). ×60.

D.—Cross section of upper part of peduncle of Little Club wheat, C. I. 4066. The collenchyma (*c*) is almost continous. ×60.

E.—A similar section from the peduncle of Marquis wheat, C. I. 3641, showing a moderate amount of sclerenchymatous tissue (*s*) in proportion to the collenchyma (*c*). ×60.

F.—Cross section of peduncle of Sonem emmer, C. I. 4402. There is a large amount of sclerenchymatous tissue (*s*) in proportion to the collenchyma (*c*). ×60.

G.—Transverse section of peduncle of Kota wheat, C. I. 5878, infected with *Puccinia graminis*. Note the small uredinium confined to a single collenchyma bundle. Lateral spread of rust is impossible because of the strands of sclerenchymatous tissue. ×45.

H.—Similar section of peduncle of Little Club wheat, C. I. 4066. Note the very large uredinium which probably has resulted from the confluence of several smaller ones. Lateral spread of infection is not restricted by sclerenchyma. ×45.

I.—Transverse section of the upper peduncle of Acme wheat, C. I. 5284, showing an almost continous band of sclerenchyma just under the epidermis. ×200.

J.—Transverse section of a portion of a typical mature leaf of Haynes Bluestem wheat showing restriction of rust mycelium to the chlorenchyma between two fibrovascular bundles. ×75.

K.—A similar leaf section from a plant of Haynes Bluestem grown in nitrogen-deficient soil. ×60.

L.—Transverse section of a leaf of Haynes Bluestem plant grown in soil containing excess nitrogen. ×60.

M.—Transverse section through the midrib of a leaf of a Haynes Bluestem plant grown in nitrogen-deficient soil. ×45.

N.—A similar section through the midrib of a leaf of a Haynes Bluestem plant grown in soil containing excess nitrogen. ×45.

THE FUNCTION OF GRIT IN THE GIZZARD OF THE FOWL[1]

By B. F. KAUPP

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INTRODUCTION

As a preliminary step in the study of the nutrition of fowls, it is necessary to determine the function of grit in the gizzard and the length of time that it will remain there and serve a useful purpose. A review of the literature of the subject discloses no record of experiments made to discover these facts.

THE PROBLEM

It is a matter of common knowledge that, since the fowl has no teeth with which to grind its food, the muscular walls of the gizzard contract upon its contents and reduce the food to fineness. The object of this investigation was to discover how long such grit is useful in the gizzard, how often it must be replenished, whether a hen constantly consumes more grit than she requires, and if so, whether the surplus is kept in the gizzard.

EXPERIMENTAL METHODS

Barred Plymouth Rock hens 2 or 3 years old were used in the experiment. They were kept in coops 18 inches square. The coops were provided with 1-inch mesh wire bottoms so that the excreta would pass through to a second floor as soon as voided. A possible reconsumption of any grit passed in the excreta was thus prevented. Hens were killed at different periods and the gizzard content examined for the grit which still remained.

Analyses of the intake and outgo of the feed and the weight of the birds were made to determine whether or not the grit content of the gizzard was sufficient for the normal physiological processes of that organ.

The feed for 365 days, the duration of the test, consisted of the regular scratch feed and dry mash used at the Station plant. The following tabulation offers a comparative study of the amount of grit contained in the gizzards of hens killed at different intervals of time. This information is differently presented in Plate 1.

[1] Accepted for publication Nov. 1, 1923.

TABLE I.—*Grit remaining in the gizzards of fowls at the end of different periods*

Hen No.	Number of days without grit.	Weight of hen at beginning of experiment.	Weight of hen when killed.	Weight of grit in gizzard.	Remarks.
		Pounds.	*Pounds.*	*Gm.*	
1	14	5.2	5.4	9.5012	Killed.
2	14	6.4	6.3	13.1136	Do.
3	21	4.5	4.8	8.3126	Do.
4	21	5.3	5.8	16.9326	Do.
5	28	4.6	4.9	11.8763	Do.
6	28	7.0	7.3	22.6531	Do.
7	36	4.8	5.7	16.4389	Do.
8	36	2.7	4.0	5.0378	Do.
9	42	7.0	4.2	8.4531	Died of sarcoma.
10	79	4.6	4.7	6.3700	Killed.
11	93	3.6	3.2	11.6341	Do.
12	120	3.8	6.2	4.9643	Do.
13	124	5.8	6.1	5.6321	Do.
14	133	5.6	4.9	4.5632	Killed by mites.
15	134	7.0	5.8	4.7532	Killed.
16	144	5.7	5.7	9.5920	Killed by mites.
17	153	7.1	5.6	6.5120	Do.
18	154	6.5	5.5	5.9633	Killed.
19	156	6.8	5.8	14.0326	Killed by mites.
20	170	(?)	(?)	9.8670	Died.[a]
21	248	6.2	6.1	2.5200	Killed.
22	270	6.3	6.6	5.0000	Do.
23	300	5.7	5.9	3.9525	Do.
24	330	5.1	6.1	1.9530	Do.
25	365	6.2	5.2	2.5610	Do.
26	395	6.7	7.1	5,8915	Do.

[a] This was a cockerel affected with partial paralysis from which it never entirely recovered. It was sent to the laboratory when it weighed about 2 pounds, and remained in the coop until it died, 170 days later.

DISCUSSION

The feed records show that the appetite of the birds having no exercise kept up fairly well.

From the experiments herein recorded it is apparent that a bird may go 365 days without grit being fed to it and still have enough remaining in its gizzard to grind its food. The grit that remained in the gizzard for 365 days appeared just as sharp as that found at the beginning of the experiment. In fact, the writer does not believe that the grinding in the gizzard of the fowl is a sharp-cutting process. Rather it appears that the food soaks more or less in the crop, depending on the length of time it remains there. It then passes from the crop through the second portion of the esophagus to the proventriculus, where it lies in an acid secretion. From the proventriculus it passes into the gizzard and there the muscles of the walls contract, forcing the soaked grain among the particles of grit and by a squeezing rotary motion reduces it to fineness. The action is like that of a ball mill. Birds hold their weight and remain perfectly healthy on either sharp or dull grit.

There is a tendency on the part of fowls to eat more grit than is essential for grinding their food. In another series of experiments the writer has found that the amount of mineral given off for the first twelve days was much greater than that taken in. Further experiments showed that this was due to the grit passed off from the gizzard. While

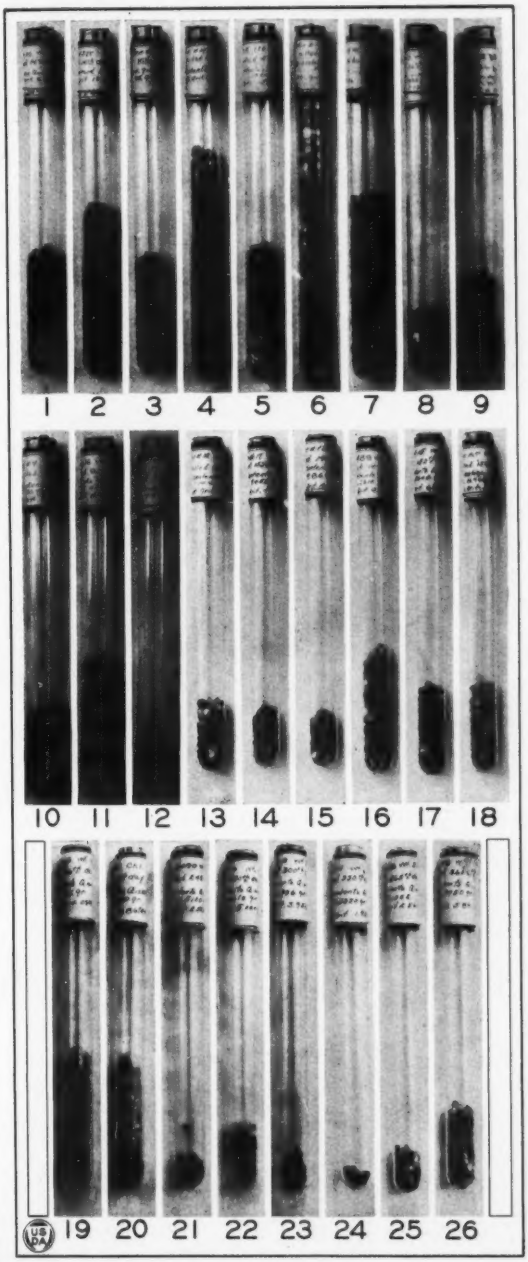
there is a tendency to pass off the excess grit and to keep a residual amount, the amount retained varies greatly in different individuals.

At the end of 365 days the gizzard of one hen contained 5.89 gm. of grit—as much as was found in a hen killed for examination on the thirty-sixth day of the experiment. A hen that died on the one hundred and fifty-sixth day had 14.03 gm. of grit, or more than that contained in three of the four gizzards of those killed on the fourteenth and twenty-first days, respectively. The healthy condition of the birds which were kept until the end of the experiment indicates that a fowl may go longer than a year without replenishing its grit.

PLATE 1

The amount of grit recovered from the gizzards of the fowls at the end of the experimental periods:

1.—Hen killed 14th day; weight of gizzard, 13 gm.; weight of grit, 9.50 gm.
2.—Hen killed 14th day; weight of gizzard, 21 gm.; weight of grit, 13.11 gm.
3.—Hen killed 21st day; weight of gizzard, 12.01 gm.; weight of grit, 8.31 gm.
4.—Hen killed 21st day; weight of gizzard, 19.03 gm.; weight of grit, 16.93 gm.
5.—Hen killed 28th day; weight of gizzard, 13.46 gm.; weight of grit, 11.87 gm.
6.—Hen killed 28th day; weight of gizzard, 28.19 gm.; weight of grit, 22.65 gm.
7.—Hen killed 36th day; weight of gizzard, 19 gm.; weight of grit, 16.43 gm.
8.—Hen killed 36th day; weight of gizzard, 7.10 gm.; weight of grit, 5.03 gm.
9.—Hen killed 42d day; weight of gizzard, 14.13 gm.; weight of grit, 8.46 gm.
10.—Hen killed 79th day; weight of gizzard 20.76 gm.; weight of grit, 6.37 gm.
11.—Hen killed 93d day; weight of gizzard, 15.43 gm.; weight of grit, 11.63 gm.
12.—Hen killed 120th day; weight of gizzard, 9.63 gm.; weight of grit, 4.96 gm.
13.—Hen killed 124th day; weight of gizzard, 14.13 gm.; weight of grit, 5.63 gm.
14.—Hen killed 133d day; weight of gizzard, 7.45 gm.; weight of grit, 4.56 gm.
15.—Hen killed 143d day; weight of gizzard, 9.86 gm.; weight of grit, 4.75 gm.
16.—Hen killed 144th day; weight of gizzard, 13.23 gm.; weight of grit, 9.59 gm.
17.—Hen killed 153d day; weight of gizzard, 11.96 gm.; weight of grit, 6.51 gm.
18.—Hen killed 154th day; weight of gizzard, 11.76 gm.; weight of grit, 5.96 gm.
19.—Hen killed 156th day; weight of gizzard 17.36 gm.; weight of grit, 14.03 gm.
20.—Hen killed 170th day; weight of gizzard, 14.93 gm.; weight of grit, 9.86 gm.
21.—Hen killed 248th day; weight of gizzard, 4.51 gm.; weight of grit, 2.52 gm.
22.—Hen killed 270th day; weight of gizzard, 10.46 gm.; weight of grit, 5 gm.
23.—Hen killed 300th day; weight of gizzard, 10.09 gm.; weight of grit, 3.95 gm.
24.—Hen killed 330th day; weight of gizzard, 6.53 gm.; weight of grit, 1.93 gm.
25.—Hen killed 365th day; weight of gizzard, 7.10 gm.; weight of grit, 2.56 gm.
26.—Hen killed 365th day; weight of gizzard, 9.97 gm.; weight of grit, 5.89 gm.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THE ASSOCIATION OF MANGANESE WITH VITAMINS [1]

By J. S. McHARGUE

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In a former paper (7)[2] data were presented to show that manganese is an essential element in the growth and normal development of autotrophic plants and functions in the synthesis of chlorophyll. The purpose of this paper is to present data which further confirm previous conclusions as to the essential character of manganese as a vital factor in life processes, to suggest a new theory concerning the nature of the vitamin factors, and to present data in support of that theory.

The observations of Takaki and the experiments of Eijkman, as quoted by Sherman and Smith (*11, p. 8–9*), show conclusively that the pericarp and germ of rice and barley grains contain a vital factor which prevents the development of beriberi in animals when the brown unpolished grains are consumed in the diet.

As early as 1854 Liebig (*4*) called attention to the fact that in the modern process of milling wheat the resulting flour contains less nutritive value than flour made from the whole grain. He states that "No single foodstuff loses its value so readily as whole grain through the modern process of milling. The whiter the flour the less nutritive value it possesses." These statements have been confirmed in many ways since the time of Liebig, and it is apparent that the pericarp and germ of wheat, barley, and rice each contains some unidentified vital factor which is necessary for the normal metabolic processes and physiological well-being in animal life.

Physiologists and biochemists everywhere are strenuously endeavoring to ascertain the function of each of the known chemical constituents of food. Since manganese is a known constituent of foods, the question arises as to whether or not this element has important functions in the metabolic processes of animal life which have been overlooked in previous investigations.

In 1914 the writer (*5*) published a paper in the Journal of the American Chemical Society, entitled "The Occurrence and Significance of Manganese in the Seed Coat of Various Seeds." In this paper it was shown that the pericarp of wheat and of a considerable number of other seeds contains much more manganese than the starchy and glutinous portions of the endosperm. It was suggested at the conclusion of the article that probably the manganese contained in the pericarp of different seeds performs an important function in the growth of plants. While this suggestion was somewhat prophetic at the time, the author has since obtained conclusive evidence that manganese does play the part of an essential element in plant growth. This fact is evidenced in data previously published (*6, 7*) and is further confirmed by the accompanying photograph of tomato plants (Pl. 1), the fruit of which is assumed to contain an important vitamin factor.

[1] Accepted for publication Nov. 19, 1923. Contribution from the Laboratory of Chemical Research of the Kentucky Agricultural Experiment Station. Published with permission of the Director.

[2] Reference is made by number (italic) to "Literature cited," p. 424.

Experiments conducted with many additional species of plants establish the fact that manganese is an essential element for the metabolic processes and the normal growth of autotrophic plants. When manganese becomes a limiting factor in the growth of plants, one of the first effects to be noted is a chlorotic condition of the young leaves and buds as they unfold—a fact which shows that this element has a function in the vital processes and is necessary for the synthesis of chlorophyll. The green leaves of plants are known to be an important source of vitamins, whereas blanched or chlorotic leaves are poor in vital factors; it has been shown also that green leaves contain more manganese than any other part of the plant, thus indicating that an interrelationship exists between chlorophyll, vitamins, and manganese.

MANGANESE IN THE PERICARP OF SEEDS

Since it has been demonstrated that the vitamin in whole grain of wheat, barley, and rice is contained in the pericarp and the germ, an examination of these cereals was made to determine the amount of manganese in the different parts of each and to show what proportion is removed in the modern processes of milling. Accordingly, samples of rice bran, rice polishings, unpolished rice grains, and polished white rice grains were analyzed for manganese. The results obtained, in parts per million of the air-dry material, are as follows:

Material:	Manganese
Rice bran	350
Rice polishings	100
Unpolished rice grains	25
Polished rice grains	10

From these results it is apparent that rice bran contains the largest quantity of manganese and the polishings contain the next largest, which is less than one-third of the amount found in the bran. The unpolished rice grains contain only one-fourteenth as much manganese as the bran and one-fourth as much as the polishings but two and one-half times as much as that found in the polished white rice. It is therefore apparent that in the process of polishing rice the greater part of the manganese is removed from the grain. It is logical to assume that the manganese contained in the pericarp and germ, and removed in the polishing, is in a different and perhaps more active combination than that contained in the more starchy and glutinous part of the endosperm. This assumption finds support in the results obtained by Eijkman (*11, p. 8–9*) and others who have found that the addition of the pericarp of the rice grain or even the bran to polished white rice prevents the development of beriberi in experiments with animals.

It has been shown by many investigators that the vitamin contained in rice bran and polishings is soluble in water, but less so in 95 per cent alcohol. The writer has examined the aqueous and alcoholic extracts from rice bran and polishings and has found that a considerable proportion (80 parts per million) of the manganese in each of these materials is soluble. The amount removed by alcohol depends upon the percentage of water contained in the alcohol. Very small amounts of manganese are soluble in 95 per cent alcohol, and the amount dissolved increases as the percentage of water in the alcohol increases, a fact in harmony with the findings that the aqueous and alcoholic extracts of rice bran and rice polishings contain the so-called vitamins.

BARLEY

The pericarp of the barley grain is slightly less pronounced than is that of unpolished rice; however, upon close observation, its presence can be detected. Five gm. of barley grains were pearled by hand to remove the pericarp and germ. Manganese was determined in the unpearled and in the pearled grains. The unpearled grains contained 20 parts per million of manganese and the pearled grains contained less than 1 part per million, thus showing that practically all of the manganese is contained in the pericarp and germ of the barley grain.

WHEAT

From the standpoint of the color of the pericarp, wheats may be divided into two general classes, red and white. Red wheats, as the name suggests, have a red or brown pericarp, while white wheats have a light-colored pericarp. Perliminary experiments indicate that a correlation exists between the color of wheats and the amount of manganese which they contain, the red varieties having a larger amount of manganese than the white. A determination of the manganese contained in one sample each of red and white wheat showed that the red variety contained 40 parts per million of manganese, and the white 20 parts per million.

It has been reported that patent flour made from red wheat, although low in the vitamin factors, contains a greater number than does a similar grade of flour made from white wheat. It is also common knowledge that red winter and spring wheats contain a larger percentage of gluten than the white varieties, a fact which prompts the suggestion that manganese may be responsible for this condition.

Samples of whole-wheat grains, wheat bran, and high-grade patent wheat flour were obtained and the manganese in each determined. The following table represents the amount of manganese in parts per million of each of the air-dried materials:

Materials:	Manganese
Wheat grains	40
Wheat bran	175
Patent flour	10

It is apparent that the bran or pericarp contains several times as much manganese as either whole grain or patent flour when equal quantities are considered. It is also clear that the patent flour contains very little manganese, the greater part having been removed in the bran and the germ. The amount of manganese found in patent flour is the same as that found in polished rice. Assuming manganese to be the vitamin factor that is removed in the milling process, we should not expect patent flour to be a preventive of beriberi—an inference which is in accord with the facts as found by other investigators in feeding experiments with animals.

From the foregoing results relative to the manganese content of the whole grains, pericarps, and the highly milled products of barley, wheat, and rice, it is evident that the greater part of the manganese is removed in the bran and polishings; it is therefore logical to assume that a compound of manganese may be the vital factor removed in the milling of these cereals.

MANGANESE ASSOCIATED WITH VITAMINS IN ANIMAL TISSUES

Various investigations concerning the vitamin content of lean meat generally concur in the conclusion that while lean meat contains some of all the vitamins it is not nearly so rich in these as are some of the glandular organs of the animal body such as the liver, kidneys, spleen, and pancreas. The writer has found no suggestions in the literature as to why these glandular organs are richer in vitamins than the lean muscular meat. Plimmer (9) states that meat consumed in small daily quantities, from 4 to 8 ounces, may not supply enough of any one factor to compensate for an absence of vitamins in the rest of the diet. However, meat consumed in very large quantities protects both from beriberi and scurvy. Plimmer (9) also states that Krogh visited Greenland to study the metabolism of Eskimos and verified the report that they sometimes eat as much as 15 pounds of meat in less than fourteen hours without ill effect. He also says that the chief or only food was boiled seal meat, liver, and blubber. The raw liver of seals and the middle epidermal layers of certain whales are regarded by the Eskimos as a sure protection against scurvy, and their value has been fully confirmed by the medical officers of Greenland. It is also stated by Plimmer that fat or muscle is not as rich in fat-soluble A factor as the fat around the internal organs, e. g., kidney suet.

Having demonstrated that manganese is necessary for the normal metabolic processes in the growth of plants, it is only natural to consider the manganese content of the animal body and whether or not this element is also necessary for the normal metabolic processes in animal life. The Department of Animal Husbandry of the Kentucky Agricultural Experiment Station furnished the organs used in the analysis shown in Table I.

TABLE I.—*Amount of manganese found in moisture-free materials*

Parts.	Hog.	Sheep.	Steer.
	P.p.m.	*P.p.m.*	*P.p.m.*
Brain	(a)	3.00	2.56
Heart	1.50	2.30	2.50
Lean meat	.50	1.25	.80
Fat	.00	Trace.	Trace.
Kidney	5.00	8.50	6.75
Liver	12.50	15.00	14.00
Pancreas	(b)	(b)	4.00

[a] Sample insufficient for a determination. [b] No sample obtained.

From the results shown in the foregoing table it is evident that the liver contains about twice as much manganese as any other part of the animal examined. The kidneys are the part next richest in manganese, while the pancreas, brain, heart, and lean meat follow in order of their manganese content. The fat of each of these animals contained no more than a trace of manganese at the most and the lean meat very little.

The findings here recorded are considerably greater than those reported by Bertrand (1), who has determined manganese in some of the same organs of these and other animals. However, he too finds the largest quantities of manganese in the liver and kidneys. These results for man-

ganese agree very closely with the findings of other investigators in showing that the liver, kidney, heart, brain, and pancreas are richer in vitamin content than other parts of the animal body, thus indicating a close parallelism between vitamin and manganese content in the parts of the animals examined. Undoubtedly manganese has a very important function to perform in the liver, kidneys, and pancreas. Since twice as much manganese was found in the liver as in any other part of the body, it is logical to assume that this accounts for the fact that the liver is also a rich source of vitamins. Several investigators report that they have found the liver of different animals a cure or preventive of the common diseases resulting from the lack of an adequate supply of vitamins.

MANGANESE IN THE LIVER OF CODFISH

Investigators have found that the liver of codfish is rich in fat-soluble A vitamin, and many efforts have been made to isolate this vitamin from cod-liver oil, but without success. According to Plimmer (*9*), fat-soluble A is synthesized in the green parts of plants, and the white leaves of cabbage contain less A factor than the green leaves. Lower plants (marine algae) containing chlorophyll synthesize this vitamin. The marine algae are eaten by small marine animals, which in turn are eaten by larger ones, eventually by codfish. The fat-soluble A factor in the codfish is chiefly concentrated in its liver.

In view of the fact that manganese was found in greater concentration in the livers of the hog, sheep, and cow than in other organs, it is reasonable to expect the liver of the codfish to contain a larger proportion of manganese than any other part of its body. A sample of cod-liver chum, the disintegrated cod-liver tissue after the steaming process, and also some of the clear supernatant cod-liver oil, were obtained from codfish caught near Portland, Me., on May 12, 1923, and each of these materials was examined for manganese. The cod-liver chum was dried at 110° C. and manganese determined in 100 gm. of the moisture-free material. The dry chum contained some oil. Four parts per million of manganese were found in the dry chum, from which it is apparent that the liver of the codfish does contain a considerable quantity of manganese. Two hundred grams of the clear, golden-colored cod-liver oil was burned by means of a wick in a platinum dish and the residue tested for manganese. Only a trace was found.

Another sample of what was claimed to be a high grade of purified Norwegian cod-liver oil which, according to the statement on the label was rich in fat-soluble A vitamin, was examined in a similar way for manganese and approximately one-tenth part per million of manganese was found. The manufacturer from whom the sample was obtained states that the potency of cod-liver oil as a source of fat-soluble A varies with the source of the oil and the treatment it has undergone in the process of refining. It is stated that cod-liver oil that has been subjected to superheated steam or has been highly refined by filtration loses its potency as a source of fat-soluble A factor.

Funk (*3*) states that he has found the crude cod-liver oil to be richer in fat-soluble A vitamin than the refined oil. He also states that Zilva and Miura (*12*), in their experiments with animals, found the crude cod-liver oil to have a fat-soluble A potency 250 times greater than butter fat. However, since it has been shown that the liver of the codfish is the richest source of the fat-soluble A vitamin and that the unre-

fined oil is richer in the fat-soluble factor than the refined oil, we can readily account for these facts by assuming that the liver is also richest in manganese; the crude oil would contain less manganese than the liver but more than the refined oil. In the process of refining the crude oil it is filtered through fuller's earth until the oil which was dark in color before filtering is changed to a golden straw color. It is apparent that the fuller's earth removes by adsorption most of the vitamin factor A, which in all probability is a colloidal form of a manganese compound. This assumption is further supported by the fact that Seidell (*10*) has made use of fuller's earth as a means of separating, by adsorption, some of the vitamin factors. However, he has neither shown nor suggested that it was a compound of manganese that was being adsorbed by the fuller's earth.

MANGANESE IN FISH ROE

One pound of fish roe was purchased at a fish market in Lexington, Ky., dried at 110° C., and 100 gm. of the moisture-free material ashed for a manganese determination. Three parts per million of manganese was found in the moisture-free roe of the fish. The livers from freshwater fish, newlights, were obtained from the same market, and 3.75 parts per million of manganese was found in the dry material of the fish livers, which is considerably less than the amount found in the livers of domestic animals. Other investigators state that the flesh of fish is poor in vitamins, whereas their roes and livers are rich in these factors, a fact in harmony with the findings of the author in regard to manganese content.

MANGANESE IN MILK

Some investigators state that milk is richest in vitamins at the beginning of the lactation and that its vitamin potency diminishes as lactation progresses. To determine whether the manganese content changes in a similar way, samples were obtained from the first colostrum of a normal cow of the station herd, taken before the calf had sucked, and from the milk of the same cow a month later. Each sample was analyzed by evaporating 1,000 gm. to dryness, ashing and determining manganese in the ash. The results are as follows:

	Colostrum, Apr. 17, 1923.	Milk, May 17, 1923.
Percentage of ash	1.154	0.711
Mn. in the ash, parts per million	20	4
Mn. in the milk, parts per million	.2	.03

The falling off in manganese content is very marked. If manganese is responsible for vitamin potency, the decline of the latter should be in a similar degree. These findings suggest that manganese is mobilized during the time the cow is not producing milk, presumably for the purpose of supplying an element that has important functions to perform during a critical period in the life of the young offspring.

MANGANESE IN THE YOLK OF EGGS

Another source of the fat-soluble A vitamin is the yolk of eggs; the white of the egg has proved to be devoid of vitamins.

One dozen fresh, viable eggs were obtained from the poultry farm of the station and hard boiled in distilled water. The eggs were then separated into three parts, shell, whites, and yolks. The whites and

yolks were dried to a constant weight at 110° C., ashed, and the manganese determined in each. The yolks contained 2.72 parts per million of manganese in the moisture-free material, and the whites did not contain a trace of manganese. This fact illustrates the association of manganese with the vitamins and further supports the idea that a compound of manganese is perhaps the vital factor in the yolk of the egg from the standpoint of foods, and also a necessary factor in the development of the embryo contained in the egg.

MANGANESE IN TOMATOES

It has been found that tomatoes are rich in vitamins. Four hundred grams of ripe tomatoes were dried to a constant weight at 110° C., ashed, and the manganese determined. In the moisture-free material 12.6 parts of manganese per million were found, corresponding to 0.62 parts per million in the tomatoes used. From the standpoint of manganese content, ripe tomatoes should have a vitamin potency three times as great as that of raw milk, which is in harmony with the results obtained with fresh tomato juice, namely, that it has a high potency as a source of vitamins.

MANGANESE IN ORANGES AND LEMONS

It has been shown that the juice of oranges and lemons contains the antiscorbutic vitamin. Cooper (*2*) has shown that the peel of oranges and lemons also contains the water-soluble vitamin, and Osborne and Mendel (*8*) have demonstrated the presence of water-soluble B in the juice of oranges.

Oranges and lemons were examined for their manganese content. The juice from 6 oranges and 12 lemons was filtered separately through cheese cloth and dried to a constant weight at 110° C., ashed, and the manganese determined. The peelings were also dried at 110° C., ashed, and the manganese determined. The results obtained for manganese, in parts per million of the moisture-free material, are as follows:

	Orange.	Lemon.
Juice	1.41	3.38
Peel	3.20	4.12

From the foregoing results it appears that the lemon contains more manganese in its juice and peel than the orange. However, most investigators assign equal values to each as a source of the antiscorbutic vitamin. The parallelism existing between manganese and vitamins occurs in oranges and lemons.

SUMMARY

Small amounts of manganese are widely distributed in nature, and it undoubtedly performs important catalytic functions in plant and animal metabolism. The author has obtained data which show conclusively that manganese is an essential element in plant economy and performs an important function, perhaps catalytic, in the synthesis of chlorophyll. The pericarp and germ of rice, barley, and wheat contain considerable manganese, but the greater part of this element is removed in the polishing and milling processes when these cereals are prepared as highly milled products for food. The value of manganese in the diet has received little consideration heretofore. It is quite logical to assume that manganese is in some way connected with the vital factor removed in highly milled rice, barley, and wheat.

Manganese occurs in largest quantities in the liver, kidney, and pancreas of animals, which leads to the assumption that it has important functions to perform in each of these organs. The occurrence of manganese in those parts of plant and animal tissues with vitamin potency suggests the idea that manganese is either directly or indirectly connected with the vital factors in these tissues.

The value of certain compounds of manganese in medicine has long been recognized, and especially so in preparations intended as cures for nervousness. Feeding experiments are now in progress to test the hypothesis that manganese is a vital factor in animal nutrition.

The fact that manganese is a necessary element in plant growth and that it is found in largest quantities in plant and animal tissues which contain the greatest vitamin potency, leads the author to assume that a relationship exists between this element and the vital factors contained in these tissues.

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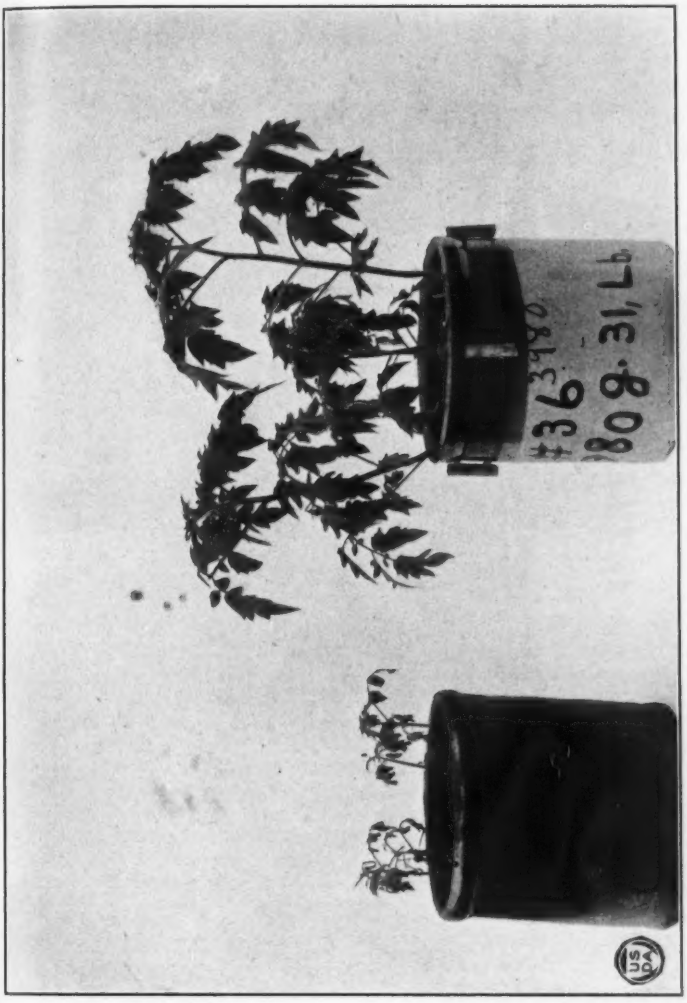
PLATE 1

All plants shown in this photograph are of the same age. The difference in size of the plants in the two pots is due to the fact that manganese was carefully excluded from the mineral nutrients and sand in the pot on the left, whereas a small quantity of a manganese salt was added to the sand in the pot on the right.